MASTER OF DENTAL SCIENCE

The impact alteration of X-ray source subject projection geometry has on the ability to detect demineralisation in occlusal cavities using digital subtraction radiography: an in-vitro study

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Glossary

ACJ	Amelo-cemental junction
ADJ	Amelo-dentinal junction
AuROC curve	Area under the receiver operating characteristic curve
CADIA	Computer-Assisted Densitometric Image Analysis
CCD	Charged-coupled devices
DALYs	Disability-adjusted life-years
DDR	Direct digital radiography
DIFOTI	Digital imaging fibre-optic transillumination
DLF	Diode laser fluorescence
DMFT	Decayed, missing and filled teeth
DR	Digital radiography
DSR	Digital subtraction radiography
ECM	Electrical conductance measurements
EIM	Electrical impedance measurements
EM	Electrical measurements
FOTI	Fibre-optic transillumination
FT	Fluorescent techniques
GBD	Global Burden of Disease
ICCC	International Caries Consensus Collaboration
ICDAS	International Caries Detection and Assessment System
IDR	Indirect digital radiography
IQR	Interquartile range

LAA	Lesion Activity Assessment
NIH	National Institutes of Health
P	Prevention
PSP	Photo-stimulable phosphor
PSPL	Photo-stimulable phosphor luminescence
Q_1	First quartile
Q_3	Third quartile
QLF	Quantitative light-induced fluorescence
ROC	Receiver operating characteristic
ROI	Region of interest
RP	Resin infiltration and prevention
SP	Sealing and prevention
SR	Samuel Rollings
WHO	World Health Organisation

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Declaration

I, Samuel Russell Rollings, am the author of this thesis, and all references cited have been consulted by me. I was the principal investigator of the research upon which this thesis is based. The thesis is a record of work that has been carried out by me, and has not previously been accepted for a higher degree.

Signed (Samuel Russell Rollings)

Date

Summary

Current recommended methods for managing carious lesions involve sealing the carious biomass within the tooth. At present, it is only possible to monitor sealed lesions over time using radiography, and digital subtraction radiography (DSR) has been demonstrated to be more accurate and reproducible compared to pairwise comparison of radiographs.

The aim of this research project was to investigate *in-vitro*, whether alteration of X-ray source subject projection geometry affected the accuracy and reproducibility of DSR for detecting demineralisation in artificially created occlusal cavities. The discriminatory ability and reproducibility of a grading system for assessing changes in the proximal relationships of teeth, on paired digital radiographs taken with horizontal variations in X-ray source subject projection geometry, was also investigated.

Digital radiographs were obtained of 40 extracted molar teeth with occlusal cavities following 7 and 15 degree horizontal, and 10 and 15 degree vertical angulation variations in X-ray source subject projection geometry in addition to 0 degrees. Following placement of demineralising solution in 19 cavities, 0 degree radiographs were taken after 12, 18 and 24 hours. Digital subtraction images were produced for each variation in angulation and length of demineralisation and scored by examiners as to their level of certainty that demineralisation of the occlusal cavity had occurred. The proximal relationships between teeth on the paired digital radiographs taken following 7 and 15 degree horizontal angulation variations in X-ray source subject projection geometry compared to 0 degrees were also scored by examiners using a grading system.

The highest accuracy for detecting demineralisation in occlusal cavities using DSR was obtained when a reproducible 0 degree X-ray projection geometry was used. However, no statistically significant reduction in accuracy was identified compared to this when digital subtraction images were produced following a 7 degree horizontal angulation variation in X-ray source subject projection geometry after 12 and 24 hours demineralisation. Intra- and inter-examiner reproducibility was moderate. When a 7 degree horizontal angulation variation in X-ray source subject projection geometry existed between paired

digital radiographs, the majority of differences observed regarding the size of inter-proximal spacing, or proximal overlapping between teeth was less than half the width of enamel. The intra- and inter-examiner reproducibility of the grading system was almost perfect.

1 Literature Review

1.1 Dental caries: the disease process and lesion formation

Cariogenic bacteria produce acid as a by-product during the metabolism of fermentable carbohydrates which can cause demineralisation of dental hard tissues, however, under suitable conditions this process can be arrested and even reversed in its early stages by remineralisation (Selwitz et al., 2007). Plaque-mediated dental diseases develop as a consequence of imbalances in the resident oral microflora due to an enrichment of oral pathogens within the bacterial community (Marsh, 1994, Marsh, 2003, Marsh and Bradshaw, 1995, Marsh and Devine, 2011). The ‘ecological plaque hypothesis’ states that ‘the selection of pathogenic bacteria is directly coupled to changes in the environment’ (Marsh, 2003) and that ‘diseases need not have a specific aetiology; any species with relevant traits can contribute to the disease process’ (Marsh, 2003).

Potentially cariogenic bacteria are often found naturally in dental plaque or biofilm on the surface of the tooth, but are weak competitors, so at a neutral pH only represent a small proportion of the total bacterial community. The levels of these potentially cariogenic bacteria are insignificant in the presence of a conventional diet and the processes of demineralisation and remineralisation remain in equilibrium. Any environmental change will affect the microbial balance. For example, an increase in the amount of fermentable carbohydrate within the environment results in the dental plaque spending more time at a low pH, which in turn favours the proliferation of more acidogenic and aciduric Gram-positive cariogenic bacteria such as mutans streptococci and lactobacilli. More acid is therefore produced, at a faster rate, favouring demineralisation of the enamel if the pH is lower than the critical value of 5.5. Net mineral loss within dental hard tissues therefore occurs at the microscopic level, which if left unchecked will develop into the white spot carious lesion. Individuals are never free of dental caries at the histological level (Kidd, 2011) as the dynamic process of enamel demineralisation and remineralisation constantly moves between net loss and net gain of tooth mineral (Frencken et al., 2012). The aim of appropriate caries management is to ensure that the fine balance between the two processes is tipped in favour of remineralisation. If this fails, further demineralisation of the tooth will occur, creating a carious lesion; the manifestation of the disease process. If this continues, the lesion will penetrate deeper into the tooth tissues and eventual microcavitation and subsequent bacterial invasion of the tooth will occur leading potentially to the need for operative intervention (Ricketts and Pitts, 2009).

1.2 Dental caries: epidemiology

Dental caries has been, and still is considered a major global oral health burden with untreated caries in permanent teeth the most prevalent oral condition in the 2010 Global Burden of Disease (GBD) Study, with a global prevalence of 35% (Marcenes et al., 2013). Untreated caries was also ranked 80th in the top 100 detailed causes of disability-adjusted life-years (DALYs) in 2010, eight places higher than recorded in 1990 (Marcenes et al., 2013). The estimated global economic impact on productivity losses during the same time period due to untreated caries in the permanent and primary dentitions were $25,138 million and $2,094 million respectively (Listl et al., 2015).

The decayed, missing and filled teeth (DMFT) index provides a lifetime measurement of dental caries in the permanent dentition and demonstrates global variation. The 2004 World Health Organisation (WHO) epidemiological databanks reported that DMFT scores for 12-year-olds were relatively high in Europe and North America (DMFT 2.6-3.0), but much lower in some African countries and South-East Asia (DMFT 1.7) (Petersen et al., 2005). However, since 1988/1989 there has been a reduction in the DMFT scores for 12-year-olds in developed countries such as England and Wales (Davies et al., 2012, Evans and Dowell, 1990) generally considered attributable to the introduction of public health measures including the effective use of fluorides and changes in lifestyle, living conditions and improved self-care practices (Petersen et al., 2005). However, the reverse has been observed in developing countries, most likely due to the increased exposure to sugars and lack of the effective use of fluorides (Petersen et al., 2005).

Dental caries is a preventable disease and significant effort is required on both a population and individual level to target prevention and reduce its prevalence.

However, complete eradication of dental caries is unlikely to occur so accurate detection methods are essential to aid its diagnosis so appropriate and effective evidence based management can be carried out.

1.3 Dental caries: management

There has been a change in philosophy around the management of dental caries (Kidd and Fejerskov, 2013) from the complete surgical excision of the carious lesion towards less invasive treatment approaches adopting the principles of ‘minimal intervention dentistry’ (Frencken et al., 2012) which aims to arrest the lesion and promote remineralisation (Tyas et al., 2000).

1.3.1 Prevention and remineralisation of carious lesions

Dental caries can be prevented by interfering with any of the environmental factors favouring the selection and increased proliferation of bacterial pathogens (Marsh, 2006). The evidence supporting the role of sugar as an aetiological factor in dental caries is indisputable, due to the number of studies, rather than the individual power of one (Sheiham, 2001, Moynihan and Kelly, 2014). One-to-one dietary interventions can change behaviour, however the evidence supporting changes in sugar consumption is less than that for changing fruit/vegetable and alcohol consumption (Harris et al., 2012).

The removal, or at least disturbance of the plaque biofilm around teeth minimises the development of carious lesions (Frencken et al., 2012), and in combination with the use of topical fluoride can slow and even arrest carious lesion progression (Fejerskov et al., 2015, Nyvad et al., 1997).

Topical fluoride changes the mineral saturation characteristics of a tooth’s surface (Fejerskov, 2004) and is incorporated into the carbonated hydroxyapatite crystalline structure of enamel during the process of demineralisation and remineralisation which decreases its solubility (De Leeuw, 2004). However, the effectiveness of fluoride to remineralise enamel is limited by the bio-availability of phosphate and calcium ions (Featherstone, 2003, Featherstone, 2006, Reynolds, 2008) and true subsurface remineralisation is rarely achievable as the remineralised surface zone acts as a diffusion barrier (Ten Cate, 2001, Ten Cate and Duijsters, 1982, Ten Cate, 1999, Ten Cate, 1990, Fejerskov et al., 2015). Cochrane reviews have demonstrated the effectiveness of fluoride for preventing dental caries, including topical fluorides such as fluoride gels (Marinho et al., 2015), varnishes (Marinho et al., 2013), toothpastes (Walsh et al., 2010) and mouthrinses (Marinho et al., 2003). For systemic fluoride, they have shown fluoridated milk reduces dental caries in primary teeth (Yeung et al., 2005), however, fluoride supplements such as tablets, drops, lozenges and chewing gums, in school children over 6-years of age are unlikely to significantly reduce dental caries in permanent teeth compared to topical fluorides (Tubert-Jeannin et al., 2011).

Preventative strategies including dietary interventions, plaque control and the use of topical fluoride should be implemented to prevent dental caries, but should also form the basis of its management (Frencken et al., 2012).

1.3.2 Management of carious lesions

All carious lesion management strategies, irrespective of the stage and/or activity of the lesion should aim to inactivate and control the disease process; preserve dental hard tissues; avoid initiating the cycle of re-restorations; and preserve the tooth for as long as possible (Schwendicke et al., 2016). The restoration of a carious lesion is only indicated if there is cavitation, and it is either non-cleansable or it can no longer be sealed (Schwendicke et al., 2016). In all other situations, carious lesions should be managed by implementing a preventative regime and considering micro-invasive treatments including sealants or resin infiltration.

1.3.2.1 Micro-invasive treatment of carious lesions

Micro-invasive treatment involves the use of acid to condition the tooth surface, which removes a few microns of tooth tissue, prior to treating the tooth surface and carious lesion with either a resin sealant or infiltration material. Although classically a highly efficacious way to prevent carious lesions forming on occlusal surfaces (Ahovuo-Saloranta et al., 2013), sealants can also be used to manage established but non-cavitated carious lesions by depriving bacteria of fermentable carbohydrate, slowing and even arresting lesion progression. Non-cavitated occlusal carious lesions extending into dentine have been effectively managed with resin sealants over a 44 month period (Fontana et al., 2014), agreeing with smaller and shorter, earlier seminal studies (Mertz-Fairhurst et al., 1986, Handelman, 1991). Sealants have also been shown to be effective for preventing the progression of proximal carious lesions (Martignon et al., 2006, Martignon et al., 2010).

Resin infiltration involves filling the porous intercrystalline spaces of the body of an enamel lesion with a low-viscosity light curable resin which seals the lesion and blocks the diffusion pathways of cariogenic bacteria inside the lesion. It is particularly useful for managing carious lesions in proximal sites as unlike sealing, no temporary tooth separation is required. Resin infiltration of proximal carious lesions in enamel and dentine is effective at reducing the risk of lesion progression (Ekstrand et al., 2010, Paris et al., 2010).

A recent Cochrane review concluded that the ‘available evidence allows us to be moderately confident that micro-invasive treatment of proximal caries lesions is effective for arresting non-cavitated enamel and dentine lesions and has a significant benefit over non-invasive interventions’ (Dorri et al., 2015).

1.3.2.2 Minimally invasive restoration of carious lesions

In the past, the management of cavitated carious lesions involved the complete surgical excision of the lesion. This approach was advocated by GV Black as he wrote in his book published in 1908 that; ‘it is better to expose the pulp of a tooth than to leave it covered only with softened dentin *(sic)*’ (Black, 1908). However, the idea of preserving tooth tissue and not removing the carious lesion as a whole is not a new one. In 1859, Tomes wrote ‘it is better that a layer of discoloured dentine should be allowed to remain for the protection of the pulp rather than run the risk of sacrificing the tooth’ (Tomes, 1859). More recent studies have shown that if the cariogenic bacteria within a carious lesion are sealed into teeth, using minimally invasive techniques, lesion progression stops and the lesion becomes inactive (Handelman et al., 1981, Mertz-Fairhurst et al., 1998, Ribeiro et al., 1999, Kuwabara and Massler, 1966).

As the complete removal of the carious lesion is not essential to arrest the cariogenic process and has undesirable adverse effects, less invasive treatment approaches have been developed which aim to arrest the carious lesion and promote remineralisation by sealing the carious biomass within the tooth. These have the advantages of being more conservative of tooth structure and reducing the chances of iatrogenic pulpal damage. Clinical studies have demonstrated a reduction in cariogenic bacteria, radiographic increase in mineral content and clinical characteristics indicating carious lesion arrest, following the selective removal of carious tissue and sealing of the carious biomass within the tooth (Maltz et al., 2002, Oliveira et al., 2006).

Historically, three broad minimally invasive techniques have been described: stepwise excavation, indirect pulp capping and partial caries removal. The stepwise excavation technique involves the removal of caries over two visits for the management of asymptomatic deep carious lesions in vital teeth where the complete removal of the carious lesion in one visit may result in pulpal exposure or damage (Leksell et al., 1996). The clinical procedure involves removing only part of the carious lesion at the first visit so that soft, carious dentine is left over the pulp and there is no risk of mechanically exposing the pulp (Schwendicke et al., 2016). Peripheral cavity dentine should be hard to allow a tight seal of the lesion and cavity using a temporary restoration and left for a period of time, during which a marked reduction in bacterial growth occurs (Bjorndal et al., 1997), and tertiary and sclerotic dentine forms (Massler, 1978). The cavity is then re-opened some months later at the second visit and further excavation of the remaining soft carious dentine is carried out prior to restoring with a definitive restoration (Schwendicke et al., 2016).

Indirect pulp capping involves removing all soft carious dentine so that only firm carious discoloured dentine is left at the base of the cavity with no knowledge of its proximity to the pulp, prior to placing a lining and definitive restoration (Prader, 1958, Eidelman et al., 1965).

There have been a variety of descriptions of partial caries removal techniques, both for the extent of carious lesion removal and for restorative procedures (Innes et al., 2016).

Due to this lack of standardisation of terminology and techniques, in particular the relative degree of caries removal carried out, it is difficult to make meaningful comparisons between different studies investigating stepwise excavation, indirect pulp capping and partial caries removal. A recent Cochrane review investigated the effects of selective, or no, caries removal in previously unrestored primary and permanent teeth (Ricketts et al., 2013). It concluded that ‘stepwise and partial excavation reduced the incidence of pulp exposure in symptomless, vital, carious primary as well as permanent teeth' and 'these techniques show clinical advantage over complete caries removal in the management of dentinal caries' (Ricketts et al., 2013).

The lack of standardisation of the terminology and techniques used by clinicians has recently led to the International Caries Consensus Collaboration (ICCC) publishing recommendations on carious tissue removal and its terminology (Schwendicke et al., 2016, Innes et al., 2016, Frencken et al., 2016). They recommended that the selective removal of carious tissue to firm dentine is the treatment of choice for shallow or moderately deep cavitated dentinal lesions, and that selective removal of carious tissue to soft dentine (for primary teeth) and stepwise removal (for permanent teeth) are the treatments of choice for deep cavitated lesions. The priorities of carious tissue removal and restoration are to preserve healthy and remineralisable tissue; achieve a restorative seal; maintain pulpal health; and maximise restorative success (Schwendicke et al., 2016).

1.3.3 Monitoring carious lesions

Despite the evidence-based change in philosophy regarding the management of carious lesions to techniques that seal the carious biomass within the tooth, and a variety of diagnostic techniques, there are no clinically proven accurate, reproducible and validated methods for monitoring the behaviour (progression, non-progression and regression) of the carious lesion over time, especially occlusal caries. Further research is therefore required.

1.4 Detection of carious lesions

1.4.1 Detection of carious lesions versus diagnosis of dental caries

The word 'diagnosis' is derived from its Greek meaning, 'through knowledge' and is a decision process informed by the collection of relevant information. The diagnosis of dental caries involves the assimilation of information gathered from the patient through accurate history taking, meticulous clinical examination with supplemental tests where indicated, an assessment of lesion activity and an individual's caries risk (Pitts, 2009).

Detection of a carious lesion is simply the identification of its presence and therefore forms only one factor in its diagnosis. In addition to detecting the presence or absence of a carious lesion, quantitative and qualitative information regarding its extent, severity and activity are also very important in the diagnostic process (Pitts, 2009).

1.4.2 Properties of the ideal carious lesion detection method

Carious lesion detection methods should have a high degree of validity, accuracy and reproducibility. In addition, due to the dynamic nature of the disease process they should also have the ability to monitor carious lesions over time especially following the implementation of preventative or micro-invasive management strategies. They should be suitable for use in general dental practice and have low technique sensitivity so identical results can be obtained irrespective of a clinician's experience of any given method and the interpretation of results. They should also be acceptable to patients, which means, they should cause minimal discomfort, be quick to carry out, universally available and economically viable to use.

1.4.2.1 Validity

The validity of any detection method or test can be defined at a basic level as the extent to which it measures what it claims to measure. In the past, four specific types of validity have been described for carious lesion detection methods; criterion, predictor, construct and content validity (Nyvad et al., 2003, Nyvad et al., 1999). As only criterion validity was used in this research project, predictor, construct and content validity will not be discussed any further.

Criterion validity requires the results of a detection method to be compared to an external 'gold standard' which represents the highest level of truthfulness that a carious lesion is either present or absent (Nyvad et al., 2003). Usually, the external 'gold standard' used is confirmation of a carious lesion (presence or absence, or extent) by histological examination of tooth sections, either visually or microradiographically (Downer, 1989). Variations may however even exist in this 'gold standard' as it relies on the subjective interpretation of what is seen on the section image (Ismail et al., 2007).

1.4.2.2 Accuracy

Sensitivity and specificity are traditional operating characteristics that are used to assess the accuracy of detection methods, describe results in a dichotomous way (Van Erkel and Pattynama, 1998) and are not affected by the prevalence of the disease being detected (Petrie et al., 2002). As many carious lesion detection methods produce either continuous quantitative, or nominal and ordinal categorical qualitative data, threshold values or decision criteria are required to convert this data into a dichotomous form to enable sensitivity and specificity to be calculated (Van Erkel and Pattynama, 1998).

1.4.2.2.1 Sensitivity

Sensitivity is defined as 'the proportion of individuals with the disease or condition of interest who are correctly detected by the test' (Petrie et al., 2002) (Table 1). If a carious lesion detection method has low sensitivity it will be less likely to detect a carious lesion when it is actually present, resulting in a false negative result.

Table 1. A 2x2 contingency table illustrating the outcomes of a detection test for a disease compared to the true presence of the disease in the population tested

	Disease present	Disease absent	Total
Test positive	True positive (TP)	False positive (FP)	TP+FP
Test negative	False negative (FN)	True negative (TN)	FN+TN
Total	TP+FN	FP+TN	FN+TN+FP+TP

Sensitivity = TP/(TP + FN)

Specificity = TN/(FP + TN)

1.4.2.2.2 Specificity

Specificity is defined as 'the proportion of individuals without the disease or condition of interest who are correctly identified by the test' (Petrie et al., 2002) (Table 1). If a carious lesion detection method has a low specificity it will falsely detect a carious lesion when it is actually not present, resulting in a false positive result.

1.4.2.2.3 Relationship between sensitivity and specificity

The ideal detection method for carious lesions would have sensitivity and specificity values of 1, which would require the probability distributions of the results to not overlap and for the chosen threshold value or decision criteria to exist between them (Van Erkel and Pattynama, 1998). However, the probability distributions of the results for carious lesion detection methods overlap which results in false negative and false positive results, affecting sensitivity and specificity which are inversely related to one another (Van Erkel and Pattynama, 1998). The balance between sensitivity and specificity for carious lesion detection methods can therefore be altered by changing the threshold value or decision criteria.

1.4.2.2.4 Describing and comparing the accuracy of detection methods

It is inadequate to use a single pair of sensitivity and specificity values at a specific threshold value or decision criteria to describe or compare the accuracy of carious lesion detection methods (Van Erkel and Pattynama, 1998). As the choice of threshold values and decision criteria are also subject to both inter- and intra-examiner variation, it is more comprehensive to describe and compare the accuracy of detection methods or tests independent of the chosen threshold value or decision criteria (Van Erkel and Pattynama, 1998). This can be achieved by carrying out receiver operating characteristic (ROC) analysis (Van Erkel and Pattynama, 1998).

1.4.2.2.5 Receiver operating characteristic (ROC) analysis

ROC analysis represents graphically the reciprocal relationship between a detection method's sensitivity and specificity at all possible threshold values. Sensitivity, on the Y-axis, is plotted against one minus the specificity on the X-axis for the various threshold values for the detection of a carious lesion (Figure 1). If categorical qualitative data is used, at least five rating categories are required to create a meaningful curve (Metz, 1989, Rockette et al., 1992).

If the probability distributions of the results of a carious lesion detection method are identical, the resulting ROC curve generated would be a straight diagonal line from bottom left to top right and is termed 'the line of no discrimination' (Figure 1). This means the detection method has no discriminative power and the area under the ROC curve (AuROC curve) is 0.5 (50% of the total area). If there is no overlap of the probability distributions of the results, the ROC curve would contain the optimal threshold value where there are no false positive or false negative results, which represents the top left hand corner of the ROC diagram. The AuROC curve would be 1 (100% of the total area). As discussed previously, in reality, this is not possible, so the ROC curves generated for carious lesion detection methods usually lie somewhere in between these two values (Figure 1). The AuROC curve therefore represents the accuracy of the detection method, and allows the accuracy between different detection methods, or examiners, to be compared using various statistical tests, each with their relative advantages and disadvantages (Swaving et al., 1996, Metz, 1989).

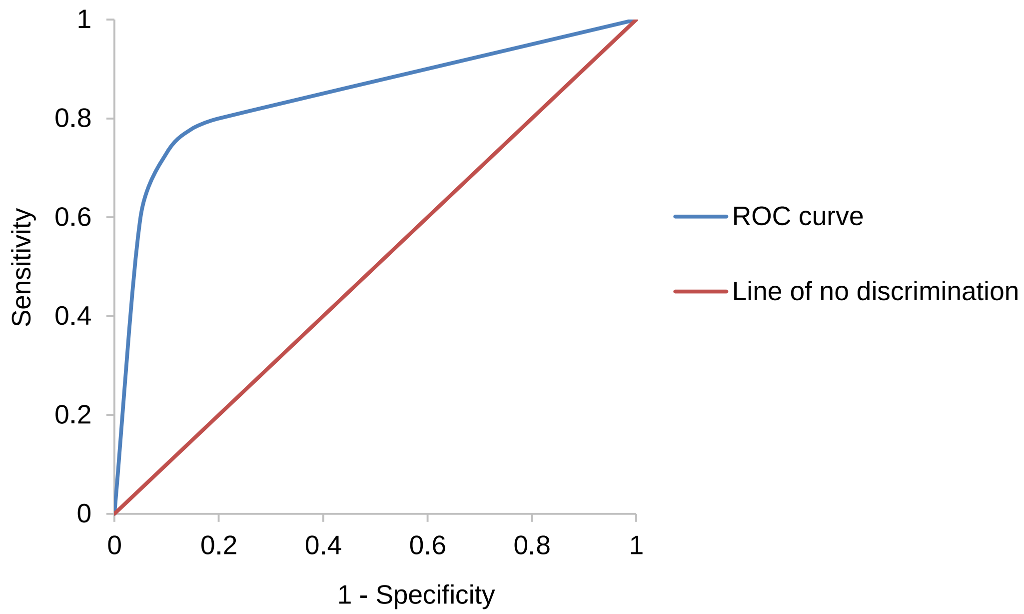


Figure 1. Receiver operating characteristic analysis

1.4.2.3 Reproducibility

Reproducibility is an umbrella term for the concepts of agreement and reliability (De Vet, 1998). The intra-examiner reproducibility of a carious lesion detection method is concerned with whether or not the results of the carious lesion detection method are reproducible in test-retest situations by the same examiner over a period of time where no clinical changes have occurred. The inter-examiner reproducibility of a carious lesion detection method is concerned with whether or not the results of the carious lesion detection method are reproducible between two or more different examiners at the same period of time where no clinical changes have occurred.

The type of data produced by the carious lesion detection method dictates the statistical test required to assess reproducibility. If continuous quantitative data is produced, intraclass correlation coefficient and Bland and Altman tests should be used (Rankin and Stokes, 1998). If categorical qualitative data is produced, percentage agreement can be calculated, however, kappa is preferred (Rankin and Stokes, 1998). Percentage agreement calculates the proportion of measurements between two examiners (inter-examiner reproducibility) or within the same examiner on different occasions (intra-examiner reproducibility) that are the same, however, it ignores the agreement that could have occurred by chance. Kappa takes into account how much agreement would be expected to be present by chance and compares this to the difference between how much agreement is actually present (Viera and Garrett, 2005). Kappa is usually used for nominal categorical qualitative data, however, it does not take into account near misses. Weighted kappa is preferred for ordinal categorical qualitative data as the degree of disagreement between examiners can be weighted, so that it attaches a greater emphasis to larger differences (Cohen, 1968).

1.4.3 Current carious lesion detection methods

Various methods have been described for detecting carious lesions, and assessing their extent, severity and/or activity, in addition to monitoring them over time. Visual, visual-tactile, fibre-optic transillumination (FOTI), electrical measurements (EM), fluorescent techniques (FT) and radiographic detection methods have all been investigated in the literature and will be discussed. Although optical coherence tomography (Ngaotheppitak et al., 2005, Amaechi et al., 2004), near infra-red imaging (Fried et al., 2005) and ultrasound (Hall and Girkin, 2004, Ng et al., 1988) have also been suggested, they are not within the scope of this literature review.

1.4.3.1 Visual and visual-tactile detection methods

Visual examination is the most common method used to detect carious lesions in clinical practice (Bader et al., 2002, Braga et al., 2010). The visual detection of non-cavitated carious lesions is based upon the optical properties of enamel and dentine and the phenomenon of light scatter which occurs due to the effect that carious lesions within a tooth's structure have on penetrating photons of light (Pretty, 2006). Teeth should be clean prior to examination as this improves the ability to detect carious lesions (Sognnaes, 1940), and due to the relative difference in the refractive indices of air and water compared to enamel, it is easier to detect enamel demineralisation if a tooth's surface is dry to reduce light scatter (Ismail, 2004). Good illumination is advised, however the use of additional white light may reduce the ability to detect carious lesions in dentine (Neuhaus et al., 2015). The benefit of magnification is debatable as although some studies suggest it improves the detection of carious lesions (Erten et al., 2005), others suggest it doesn't (Mendes et al., 2006, Akarslan and Erten, 2009).

As the visual detection of carious lesions can be unreliable due to its subjective nature (Braga et al., 2010) and the inconsistencies that exist between clinicians' interpretation of the clinical characteristics of carious lesions (Bader et al., 2002), a large number of criteria systems have been suggested for the visual detection of carious lesions (Ismail, 2004). However, the majority are ambiguous, do not measure the different stages of the caries process or its activity, don't apply to all surfaces of a tooth and don't include detection of carious lesions associated with restorations or sealants (Ismail, 2004, Bader et al., 2002). Therefore, in 2002 a group of caries researchers, epidemiologists and restorative dentists proposed a new validated system for detecting and assessing carious lesions at the International Consensus Workshop on Caries Clinical Trials in Loch Lomond, Scotland. It was named the International Caries Detection and Assessment System (ICDAS) and the philosophy and rationale for its development have been described (Pitts, 2004). In 2005, ICDAS was improved by exchanging codes to ensure the system reflected increasing severity of lesions which resulted in the creation of ICDAS-II (Ekstrand et al., 2007, Ismail et al., 2007). The accuracy of visually detecting carious lesions is improved when detailed and validated indices are used, in particular ICDAS, as it has been demonstrated to be statistically significantly more accurate for detecting initial and advanced occlusal caries in primary teeth, and advanced occlusal caries in permanent teeth compared to when no criteria system is used (Gimenez et al., 2015b).

A recent systematic review and meta-analysis (Gimenez et al., 2015b) investigated the accuracy of visual inspection for detecting carious lesions, and concluded that the overall accuracy was similar to that previously reported in systematic reviews investigating radiographic (Bader et al., 2002) and fluorescence-based (Gimenez et al., 2013) detection methods. The systematic review and meta-analysis by Gimenez *et al.* in 2015 (Gimenez et al., 2015b) also identified that visual inspection tends to have a greater specificity compared to sensitivity for detecting carious lesions, which is the opposite to radiographic and fluorescence-based detection methods which tend to have higher sensitivity compared to specificity (Bader et al., 2002, Gimenez et al., 2013). Historically, when carious lesions were routinely managed by removing the lesion in its entirety and the placement of a restoration, it had been stated that it was more appropriate for carious lesion detection methods to have a high specificity, even at the expense of a lower sensitivity, as this reduced the number of false-positive results that could result in the overtreatment of a slowly progressing disease (Downer, 1989). However, following the change in philosophy for managing carious lesions, especially for non-cavitated carious lesions towards the use of preventative and micro-invasive methods, the consequences of overtreatment are less significant. The recent systematic review and meta-analysis by Gimenez *et al.* in 2015 (Gimenez et al., 2015b) concluded that visual inspection alone appears to be effective for detecting carious lesions, without having to use adjunct radiographic or fluorescence-based detection methods. However, most studies investigating the accuracy of visual inspection for detecting carious lesions have not been directly related to the improvement of patients' oral health, and not clinically relevant or useful (Baelum, 2010) so it has been recommended that future studies investigating carious lesion detection methods should evaluate patient-centred and clinically relevant outcomes in addition to accuracy (Gimenez et al., 2015a).

The use of tactile examination using probes, in combination with visual examination has been questioned because of the occurrence of iatrogenic damage, with probing-related defects, enlargements and damage to tooth surfaces with early carious lesions having been reported (Kühnisch et al., 2007, Ekstrand et al., 1987). However, detecting the surface texture of carious lesions can be useful in assessing carious lesion activity and the Lesion Activity

Assessment (LAA) involves tactile examination, by dragging a ball ended probe across the surface of the lesion which doesn't cause any damage and allows the operator to detect whether the surface of the tooth is rough (active enamel carious lesion) or smooth (inactive enamel carious lesion scar) (Ekstrand et al., 2007).

1.4.3.2 Fibre-optic transillumination (FOTI)

FOTI uses the phenomenon of light scatter to detect carious lesions, which occurs due to the effect that the demineralisation of enamel and dentine has on penetrating photons of light. It enhances these optical properties by shining a high intensity white light through the tooth which results in areas of demineralisation appearing visually as a shadow (Pretty, 2006). FOTI has been claimed to be particularly useful for detecting proximal carious lesions (Peers et al., 1993, Deery et al., 2000, Davies et al., 2001, Mialhe et al., 2009), however, most likely due to the subjective nature of interpreting FOTI, large variations in sensitivity (Bader et al., 2002) and inter-examiner reproducibility (Hintze et al., 1998) have been reported. A recent systematic review investigating non-cavitated carious lesion detection methods concluded that the strength of evidence for the use of FOTI as a method for detecting carious lesions was poor due to the limited number and average quality of studies (Gomez et al., 2013). To attempt to overcome the subjective nature of interpreting FOTI, algorithms have been developed to provide quantitative characterisation of a carious lesion (Schneiderman et al., 1997) and to enable it to be used to monitor carious lesions over time, digital imaging fibre-optic transillumination (DIFOTI) has been introduced which produces a digital image which can be saved.

1.4.3.3 Electrical measurements (EM)

EM detect carious lesions by assessing either electrical conductance or electrical impedance of the tooth tissue. Tissues with high concentrations of fluid and electrolytes are more conductive than tissues with low concentrations. Electrical conductance measurements (ECM) detect the increase in conductance associated with carious lesions compared to sound tooth tissue that occurs following the demineralisation of enamel and dentine and increase in fluid filled pore volume. The impedance of tissues is determined by their molecular composition and refers to the degree that they resist electric current flow. Electrical impedance measurements (EIM) detect the lower electrical impedance that is associated with carious lesions compared to that of sound tooth tissue. The systematic review by Bader *et al.* in 2002 (Bader et al., 2002) which investigated the performance of methods for identifying carious lesions reported that it is difficult to compare the results from different studies using different devices that measure EM as validation methods and the severity of carious lesions differ. Despite these limitations, EM appeared to have a lower specificity for detecting occlusal carious lesions than the use of FOTI, visual and visual-tactile methods despite having a higher sensitivity (Bader et al., 2002).

1.4.3.4 Fluorescent techniques (FT)

Fluorescence is a form of luminescence and is the emission of light by an object that has absorbed light or other electromagnetic radiation. Quantitative light-induced fluorescence (QLF) and diode laser fluorescence (DLF) have both been used to aid the detection of carious lesions.

The source of fluorescence in teeth when QLF is used is believed to be from the fluorophores contained within the amelo-dentinal junction (ADJ) (van der Veen and de Josselin de Jong, 2000, Pretty, 2006). Demineralised tooth tissue demonstrates reduced fluorescence compared to sound tooth tissue due to the scattering effect of the carious lesion, which results in less excitation light reaching the ADJ, and back scattering of fluorescence from the ADJ by the carious lesion (Pretty, 2006). QLF devices transmit visible blue light to a tooth using a handpiece and the resultant fluorescence is detected and measured. QLF can be used to image all tooth surfaces with the exception of proximal sites. Unlike QLF, the source of fluorescence in teeth when DLF is used is believed to be from bacterial porphyrins, hence DLF measures the degree of bacterial activity (Pretty, 2006). DLF devices use a diode laser to emit a red excitation light to the tooth. The resultant induced fluorescence from the bacterial porphyrins is detected and measured. DLF can be used to detect carious lesions on occlusal and smooth surfaces, in addition to proximal sites.

A systematic review and meta-analysis by Gimenez *et al.* in 2013 (Gimenez et al., 2013) investigated the accuracy of all fluorescence-based methods for detecting carious lesions on all tooth surfaces of both permanent and primary teeth, compared to the previous systematic review by Bader and Shugars in

2004 (Bader and Shugars, 2004) which was limited to laser fluorescence methods only. Despite the heterogeneity of studies, Gimenez *et al.* (Gimenez et al., 2013) demonstrated that all the fluorescent techniques had similar accuracy for detecting occlusal and proximal carious lesions on permanent and primary teeth, however, accuracy was greater for detecting more advanced carious lesions. Fluorescent techniques have a higher sensitivity than visual examination for detecting more advanced occlusal carious lesions, however, the specificity tends to be lower (Gimenez et al., 2013, Bader and Shugars, 2004).

1.4.3.5 Radiographic detection methods

The use of radiographic techniques for detecting and monitoring carious lesions is discussed in more detail in section 1.7 of this literature review.

1.4.4 Monitoring carious lesions following their management

Visual, visual-tactile, FOTI, EM and FT methods can all be used to detect carious lesions, however, depending on how a carious lesion is managed, not all of these methods are suitable for monitoring progression, non-progression or regression of carious lesions over time. For management using preventative strategies alone (e.g. dietary interventions, plaque control and topical fluoride), these methods are suitable. However, these methods become redundant if a carious lesion is sealed, using either a sealant, resin infiltration or by placing a restoration as part of stepwise excavation or following the selective removal of carious tissue. In this situation radiographic methods have to be used to monitor the behaviour of the carious lesion over time.

1.5 Dental Radiography

X-rays were discovered in 1895 by Wilhelm Conrad Roentgen and were used in dentistry for diagnostic imaging as early as 1896 (Langland and Langlais, 1997). Since then significant advances have been made in the field of oral and maxillofacial radiology (Langland and Langlais, 1995). One of the most recent advances following the advent of computers has been the development of digital radiography (DR), which demonstrates a number of significant advantages over conventional film-based radiography (van der Stelt, 2008).

1.5.1 Digital radiography (DR)

The early stages of DR involved scanning, or digitising images from conventional radiographic films which enabled them to be viewed on a computer monitor which is known as indirect digital radiography (IDR) (Analoui and Stookey, 2000). Electronic image receptors were introduced in the 1960s which enabled the development of direct digital radiography (DDR) (Analoui and Stookey, 2000). The use of DDR in dental practice has become increasingly common through two systems; those using charged-coupled devices (CCD) and those using photo-stimulable phosphor (PSP) plates.

1.5.1.1 Image production in DR

Conventional X-ray equipment is used to irradiate the real time solid-state sensors used in CCD. The amount of X-radiation that reaches the different points of the surface of the sensor is converted into a signal, the strength of which determines the grey-scale value of each pixel in the digital image. PSP plates produce direct digital images through a process termed photo-stimulable phosphor luminescence (PSPL). Conventional X-ray equipment is used to irradiate the coating of the PSP plate which stores energy within it. A laser stimulates the coating of the plate which releases the stored energy as light and a scanner connected to a computer records the amount of light emitted from different points of the surface which determines the grey-scale value of each pixel in the digital image.

The digital image is comprised of binary digits (bits) positioned in rows and columns which form a matrix and each point in the matrix is termed a picture element (pixel). The spatial resolution of the digital image is dictated by the pixel size and the resolution of DR systems currently ranges from 20µm to 50µm (Analoui and Stookey, 2000). Each pixel is characterised by three numbers, its x- and y-coordinate within the matrix and its grey-scale value. The grey-scale value corresponds to the X-ray intensity at that location during the exposure of the sensor and the number of grey-scale shades within a digital system therefore determines the radiographic image contrast. Typically, a standard matrix in intra-oral radiography would have a matrix size of 512 x 512 pixels with 256 shades of grey, from 0 (black) to 255 (white).

The quality of any radiographic image is related to sharpness (which is defined by contrast and blur) and noise (Haus, 1985). Contrast describes the difference in magnitude of the optical densities of the structures of interest and their surroundings and blur describes the lateral spread of a structural boundary (Wenzel, 1993). Noise reflects all the factors that have disturbed the signal of interest such as anatomic background structures (referred to as anatomic noise) and those caused by receptor mottle (referred to as random noise) (Wenzel, 1993). The radiographic image quality of a conventional radiograph is determined by factors related to its exposure, sensitometric properties of the film and processing procedures, so once a conventional film has been processed the quality of the radiographic image has been determined and cannot be changed (van der Stelt, 2008). In contrast, it is possible to manipulate digital radiographic images once they have been acquired via image processing which allows the image quality to be altered.

1.5.1.2 Image analysis

Image analysis refers to the ability of digital radiographic software packages to carry out certain calculations and extract specific information from digital radiographic images without altering the image (van der Stelt, 2008). Image analysis tools include ones that can measure angles or distances, however they have to be calibrated as they do not account for any magnification or distortion of the radiographic image that may have occurred due to the geometric relationship between the X-ray beam, sensor and patient (van der Stelt, 2008).

1.5.1.3 Image processing

The manipulation of a digital radiographic image after it has been acquired is termed image processing and involves the application of mathematical operations to alter pixel values over the whole digital radiographic image.

1.5.1.3.1 Image quality

The blur, noise and contrast of a digital radiographic image can be altered to improve its quality (van der Stelt, 2008). Underexposed or overexposed digital radiographic images can be corrected by adjusting the contrast and density manually, or automatically by applying standard gamma optimisation procedures which distribute evenly the grey-scale values of the pixels in the digital radiographic image over the full grey-scale range that is available (van der Stelt, 2008, Wenzel, 1993).

1.5.1.3.2 Contrast enhancement

Alteration of the contrast and density of a correctly exposed digital radiographic image is termed contrast enhancement and is related to image sharpness (Wenzel, 1993). It has been reported that increasing the contrast and density of a digital radiographic image enhances the ability to detect carious lesions (van der Stelt, 2008, Wenzel et al., 1991, Mouyen et al., 1989, Verdonschot et al., 1992, Wenzel et al., 1993). Contrast enhancement can also improve the ability to detect carious lesions using digitised images generated from conventional films of poor quality, compared to viewing the original conventional films used (Wenzel and Fejerskov, 1992).

1.5.1.3.3 Edge enhancement

Edge enhancement converts contrast gradients within digital radiographic images into a texture that is visible as a shape which effectively sharpens the edges of imaged structures making it easier to identify boundaries (Kal et al., 2007). Edge enhancement has been demonstrated to improve the ability to detect and assess occlusal carious lesions on digital radiographic images when used in combination with contrast enhancement, compared to viewing conventional films (Wenzel et al., 1990, Wenzel et al., 1991). It may not however be as effective as contrast enhancement when used alone as the improvement in accuracy for detecting occlusal carious lesions has been credited to the effect of contrast enhancement rather than edge enhancement (Wenzel and Fejerskov, 1992).

1.5.1.3.4 Zoom

The alteration of the size of digital radiographic images displayed on computer monitors is commonly termed as the ability to zoom in and out. If a digital radiographic image is enlarged to a size greater than its original resolution its diagnostic value is reduced. This is because either interpolation, which is the construction of new pixels within a known range by analysing the neighbouring pixels, or duplication of the rows and columns of pixels is carried out which can both result in the appearance of artefacts (van der Stelt, 2008).

1.5.1.3.5 Digital subtraction

Digital subtraction involves obtaining two digital radiographs taken of the same object, but on two separate occasions (using identical or similar X-ray projection geometry and densitometric parameters), and digitally superimposing them so that the grey-scale values of the corresponding pixels can be subtracted from each other. This eliminates the identical structures present in the two digital radiographic images of the same object, and enables visualisation and detection of any differences that exist between them on the resulting image which is called a digital subtraction image. It is often necessary to apply patch minimisation and density normalisation to the two digital radiographic images prior to subtracting them from each other, to standardise any geometric or densitometric variations that may exist between them. Patch minimisation attempts to correct geometric misalignments that exist between pairs of digital radiographs, and density normalisation attempts to correct differences in densitometric properties (density and contrast) that exist between pairs of digital radiographs. Patch minimisation, density normalisation and digital subtraction will be discussed further in the next section.

1.6 Subtraction radiography

Prior to DR, subtraction images were obtained from conventional radiographs by photographic methods. Following the introduction of IDR, the alignment and superimposition of digital radiographic images was initially carried out by digitising one of the conventional films and manually superimposing it over the other conventional film using a micromanipulator and real-time continuous signal grabbing under a video camera, prior to digitising the second conventional film (Grondahl et al., 1983). The development of DDR has made this process a lot easier as two digital radiographic images can simply be aligned and superimposed over each other using corresponding reference points. Digital subtraction software is then used to subtract the grey-scale values of the corresponding pixels of the two digital radiographic images to produce a digital subtraction image (van der Stelt, 2008).

1.6.1 Digital subtraction radiography (DSR)

Reliable DSR requires that the two digital radiographic images used to produce the digital subtraction image have geometric and densitometric standardisation between them, i.e. the two digital radiographic images have been generated using a reproducible X-ray projection geometry and have identical grey-scale values (van der Stelt, 1993, van der Stelt, 2008, Christgau et al., 1998, Eberhard et al., 2000, Wenzel, 2002, Dove and McDavid, 1992, Haiter-Neto et al., 2005, Rudolph et al., 1987, Grondahl et al., 1984, Hausmann et al., 1991, Ruttimann et al., 1981, Wenzel et al., 1993, Janssen and van Aken, 1989). Geometric and densitometric variation affects the quality of the resultant digital subtraction image due to the generation of structured noise in the image. Digital subtraction software packages therefore use patch minimisation and density normalisation processes to attempt to standardise any geometric or densitometric variations that may exist between digital radiographic images prior to digitally subtracting them to produce the digital subtraction image.

1.6.1.1 Geometric standardisation

Two main sources of geometric error exist when comparing two radiographs. Firstly, a difference in the relationship between X-ray beam and subject which results in a source-subject error and secondly, a difference in the relationship between the subject and the imaging medium which results in a subject-film error (Jeffcoat et al., 1987). A source-subject error results in a different 2-dimensional representation of a 3-dimensional subject, however, a subject-film error results in a different 2-dimensional projection of the same 3-dimensional data (Ellwood et al., 1997). It has been stated that ‘it is impossible to manipulate images which have a source-subject error to try to overcome this distortion without having additional information regarding the 3-dimensional relationship between structures’ (Ellwood et al., 1997). However, ‘subject-film errors can be overcome as the relationship between the two images can be defined mathematically and one of the images transformed into the same projection geometry as the other’ (Ellwood et al., 1997) using patch minimisation (Jeffcoat et al., 1984, Webber et al., 1984).

Although subject-film errors can be compensated for using patch minimisation processes, other techniques are required when taking digital radiographs to reduce source-subject errors. Prior to the development of subtraction radiography, the importance of standardising X-ray projection geometry for aiding the comparison of two different radiographic images of the same object had been identified. The first intra-oral film holder was described only four years after the discovery of X-rays and was used to aid with periapical radiography (Kells, 1899). Since then various devices have been suggested and research carried out to identify the best method to standardise intra-oral X-ray projection geometry.

1.6.1.1.1 Devices for standardising intra-oral X-ray projection geometry

A wide variety of devices have been developed in an attempt to standardise intra-oral X-ray projection geometry, all of which essentially comprise the same components; a film holder, bite block and beam aiming device which are connected together but differ in design. Film holders stabilise the position of the radiographic film or sensor in a fixed position. Bite blocks enable the device to be stabilised in relation to the teeth and can be customised, however reseating customised bite blocks fully can be difficult over time due to either distortion of the block or material used to customise it, movement or extraction of the supporting teeth or modification of the occlusal surfaces of teeth with restorations. Beam aiming devices are connected to the film holder and bite block and allow standardisation of the X-ray tube head position to the device. The majority of beam aiming devices use a non-rigid connection, however a rigid or fixed connection between the beam aiming device and X-ray tube head is possible.

A number of studies have investigated the reproducibility of a variety of customised commercially available or custom fabricated devices for standardising intra-oral X-ray source subject projection geometry (Allen et al., 1994, Duckworth et al., 1983, Hausmann et al., 1995, Janssen et al., 1989, Rudolph and White, 1988) (Table 2). The use of customised bite blocks with commercially available devices can limit intra-oral X-ray source subject angulation errors to less than 3 degrees in both the vertical and horizontal dimension over a 6-month period (Duckworth et al., 1983, Rudolph and White, 1988). Although custom fabricated non-commercially available devices have demonstrated slightly lower angulation errors over the same or slightly longer time periods (Allen et al., 1994, Hausmann et al., 1995, Janssen et al., 1989), it is necessary to investigate what evidence there is to suggest that this significantly improves the diagnostic quality of the digital subtraction images produced.

Table 2. Studies investigating the reproducibility of customised commercially available and custom fabricated devices for standardising intra-oral X-ray source subject projection geometry

Study	Device(s)	Duration	Mean average source-subject angulation error
Duckworth *et al.* (1983)	3 customised VIP Film Holders™ (Dentsply Rinn, Illinois) - Polyether bite block - Silicone bite block - Acrylic bite block	6 months	VIP Film Holder™ (Dentsply Rinn, Illinois) using a polyether bite block (Polyjel, L.D. Caulk Company, Delaware) - Horizontal = 1.25 degrees +/- 0.93 - Vertical = 2.39 degrees +/- 2.23 (P<0.001 compared to all other devices tested)
Rudolph & White (1988)	7 Rinn XCP Instruments™ (Dentsply Rinn, Illinois) - Standard bite block - 6 different customised bite blocks using different types of acrylic, compound, polyether and silicone 1 Customised VIP Film Holder™ (Dentsply Rinn, Illinois) - Polyether bite block (as described by Duckworth *et al.* (Duckworth et al., 1983))	6 months	Rinn XCP Instrument™ (Dentsply Rinn, Illinois) with a silicone bite block (Regisil, L.D. Caulk Company, Delaware) - Horizontal = 1.34 degrees +/- 0.63 - Vertical = 2.04 degrees +/- 0.82 (P<0.05 compared to standard Rinn XCP Instrument™ and customised VIP Film Holder™)
Janssen *et al.* (1989)	2 custom fabricated paralleling devices with bite blocks - Unilateral acrylic bite block - Bilateral acrylic bite block	1 year	Bilateral bite block - 1.15 degrees Unilateral bite block - 1.47 degrees (P<0.07 therefore no statistically significant difference)
Allen *et al.* (1994)	1 custom fabricated stent based system (as described by McHenry *et al.*(McHenry et al., 1987) which was based on the device described by Rosling *et al.* (Rosling et al., 1975)) - Full arch custom acrylic stent with a stainless steel film holder attached to it, which is rigidly attached to the X-ray tube with a brass bar	6 months	1.55 degrees Errors < 2 degrees 75% of the time
Hausmann *et al.* (1995)	1 custom fabricated electronically guided, force-sensitive sensor-based alignment system - Full arch custom acrylic stent - Alignment arch and ring - Matching ring for X-ray tube with 3 force-sensitive sensors and electric monitor	6 months	Anterior sites - 1.95 degrees +/- 1.17 - Errors <2 degrees 68% of the time Posterior sites - 1.58 degrees +/- 1.07 - Errors <2 degrees 68% of the time

1.6.1.1.2 The effect of variations in X-ray source subject projection geometry on the quality of intra-oral digital subtraction images

Four *in-vitro* studies have investigated the effect that variations in X-ray source subject projection geometry have on the ability to detect changes using intra-oral DSR. However, these were investigating changes in bone (Davis et al., 1994, Grondahl et al., 1984, Rudolph et al., 1987, Wenzel, 1989). All of the studies demonstrated that as the size of the variation in X-ray source subject projection geometry increased, the accuracy of the resulting digital subtraction images for detecting changes decreased (Davis et al., 1994, Grondahl et al., 1984, Rudolph et al., 1987, Wenzel, 1989). Three studies demonstrated that DSR can accurately detect artificially created bony defects up to $1mm^3$ (Grondahl et al., 1984), and increases in thickness of bone of 0.42mm (Rudolph et al., 1987) and 0.55mm (Wenzel, 1989) with variations in X-ray source subject projection geometry of up to 3 degrees. This degree of X-ray source subject error is within that achieved using the intra-oral devices reported by Duckworth *et al.* (1983) and Rudolph and White (1988) for standardising X-ray source subject projection geometry. However, one study suggested that variation in X-ray source subject projection geometry of 2 degrees or more resulted in a statistically significant reduction in the sensitivity of DSR for detecting $1.105mm^2$ bony defects, compared to viewing digital subtraction images that had been produced using a reproducible X-ray source subject projection geometry (Davis et al., 1994).

Periodontal bony defects are unlikely to present clinically with as well demarcated edges as those created artificially in these four *in-vitro* studies, and as detection accuracy is dependent on the size of the difference that actually exists and the required difference that is required to be detected, it is difficult to know if these results can be applied to the clinical detection of periodontal bony changes over time. To date there have not been any studies that have investigated the effect that variation in source subject angulation has on the accuracy of DSR to detect demineralisation within teeth, however, horizontal changes in source subject angulation affects the assessment of the extent and depth of proximal carious lesions when viewing conventional radiographic images (Chadwick et al., 1999, Sewerin, 1981b).

1.6.1.1.3 Patch minimisation

Patch minimisation uses semi-automated functions; user-chosen reference points and mathematical algorithms, to attempt to correct geometric misalignments that exist between pairs of digital radiographs due to subject-film errors (Dunn and van der Stelt, 1992, Dunn et al., 1993). The Compare Software (Dental Health Unit, University of Manchester, UK), which has been used in this research project and a number of other studies investigating the use of DSR, runs as a plug-in to Image Tool (version 1.23, University of San Antonio, Texas) and uses a semi-automated registration process to carry out patch minimisation. It requires the operator to manually select two reference points, usually the mesial and distal amelo-cemental junction (ACJ) of the tooth of interest, on both the ‘before’ (Figure 2) and ‘after’ (Figure 3) digital radiographic images to enable a preliminary warp to be carried out of the two digital radiographic images. Following the manual selection of the region of interest (ROI) on the ‘before’ digital radiographic image by drawing a patch (Figure 4), an automated mathematical algorithm finds the corresponding points of least difference on the after digital radiographic image. The mathematical algorithm automatically carries out translation, rotation, scaling and shear of the patch to define the relationship of the two digital radiographic images to enable a matrix transformation to be carried out to warp the before digital radiographic image into the same geometric alignment as the after digital radiographic image (Ellwood et al., 1997).

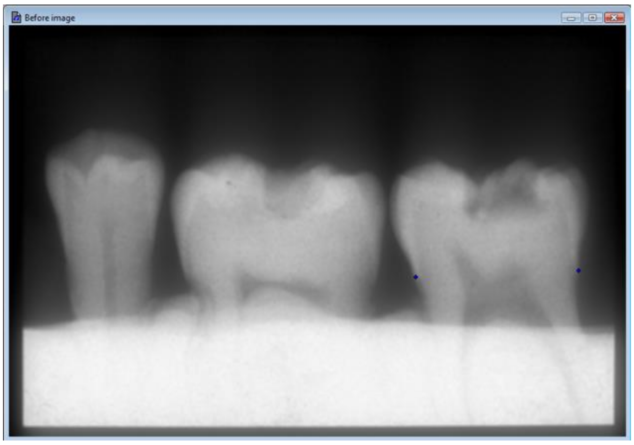


Figure 2. Manual selection of mesial and distal ACJ for the lower left second molar tooth on ‘before’ digital radiographic image

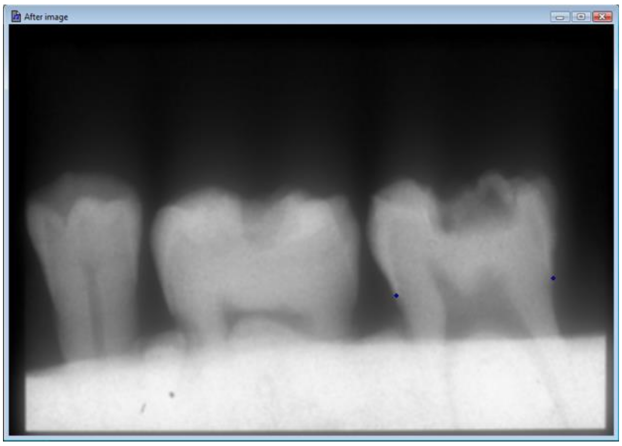


Figure 3. Manual selection of mesial and distal ACJ for the lower left second molar tooth on 'after' digital radiographic image

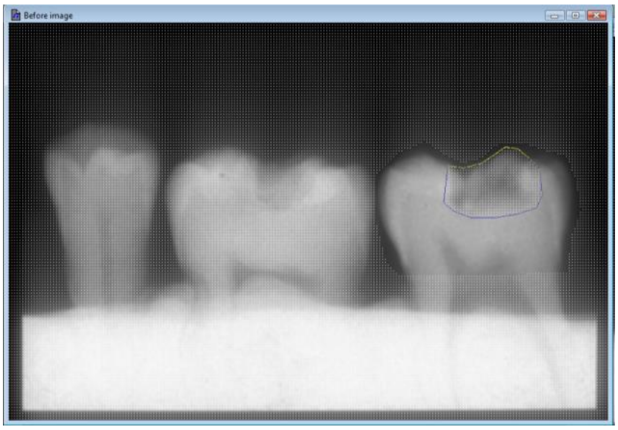


Figure 4. Manual selection of the ROI for the lower left second molar tooth on the 'before' digital radiographic image by drawing a patch

1.6.1.2 Densitometric standardisation

Differences in densitometric properties (density and contrast) can exist between direct digital radiographic images due to differences in the exposure parameters, sensors or X-ray projection geometry used to generate them, or differences in anatomical structures. Any differences in indirect digital radiographic images may also be due to variations in radiographic film or processing procedures.

1.6.1.2.1 Density normalisation

Density normalisation attempts to correct differences in densitometric properties that exist between two digital radiographic images (Wenzel, 1989, Ohki et al., 1988, Ruttimann et al., 1981, Ruttimann et al., 1986, van der Stelt, 2008). Density normalisation procedures densitometrically adjust the geometrically transformed after digital radiographic image into concordance with the before digital radiographic image (Ellwood et al., 1997). Mathematical algorithms have been developed to derive the required grey-scale transformations and parametric methods were initially proposed (Ruttimann et al., 1981). However, a modification of the non-parametric histogram normalisation method to modify pixel values was shown to yield statistically significant better results in terms of standard deviations in the digital subtraction images (Ruttimann et al., 1986). Further research has supported this as a non-parametric algorithm has been found to be statistically significantly better than algorithms based on linear approximation (Fourmousis et al., 1994a).

1.6.1.3 Digital subtraction

As discussed in section 1.5.1.3.5, DSR software superimposes the two digital radiographs that have undergone patch minimisation and density normalisation on top of one another. The software then identifies the grey-scale values of the corresponding pixels on the before and after image, and subtracts them from one another to produce a digital subtraction image. To ensure that the digital subtraction image that is produced is in the centre of the grey-scale range, 127 is added to each of the resulting grey-scale values that form the digital subtraction image. This means that if there has been no change in the grey-scale value of the corresponding pixels on the two digital radiographs, then that pixel has a grey-scale value of 127 on the digital subtraction image; the middle of the grey-scale range. Any areas of the digital subtraction image that appear lighter than this (grey-scale value >127) indicate that an increase in radiodensity has occurred for that corresponding pixel, and any areas that appear darker than this (grey-scale value <127) indicate that a decrease in radiodensity has occurred for that corresponding pixel. Qualitative assessments can therefore be made using digital subtraction images, however, studies have also demonstrated that quantitative assessments can be made by calibrating optical densities against a known standard (Vos et al., 1986, Fourmousis et al., 1994b, Ellwood et al., 1997, Bragger et al., 1998, Jeffcoat and Reddy, 1993).

1.6.1.3.1 Contrast enhancement

Contrast enhancement augments small differences in grey-scale values which may not be apparent if the digital subtraction image shows very little grey-scale variability. This theoretically aids visualisation of smaller differences (Versteeg and van der Stelt, 1995). Linear contrast enhancement increases all density differences equally including those attributed to various sources of noise. Logarithmic contrast enhancement does not increase all density differences equally, instead, it increases the differences in grey-scale values closer to 127 to a greater extent than differences in grey-scale values closer to 0 (black) or 255 (white).

1.6.1.4 DSR and its use in dentistry

At present, the use of DSR in dentistry is primarily limited to research. Studies have investigated its use for assessing and monitoring periodontal bone levels (Hausmann et al., 1988, Schmidt et al., 1988, Wenzel et al., 1992, Grondahl and Grondahl, 1983), assessing and monitoring peri-implant bone levels (Engelke et al., 1990, Bragger et al., 1991, Bittar-Cortez et al., 2006, Jeffcoat et al., 1995), monitoring peri-radicular healing following endodontic treatment (Nicopoulou-Karayianni et al., 2002) and aiding the identification of victims in forensic dentistry (Wenzel and Andersen, 1994). Its use investigating detection and monitoring of dental carious lesions is discussed in section 1.8.

1.7 The detection and monitoring of carious lesions using radiography

The decreased mineral content of enamel, and subsequently dentine within a carious lesion reduces the attenuation of an X-ray beam as it passes through it, compared to non-carious enamel and dentine. This produces a relative radiolucent area on the radiographic film or digital radiographic image which corresponds to the presence of the carious lesion. The greater the relative decrease in mineral content of the carious lesion compared to the adjacent non-carious enamel and dentine, the more radiolucent the lesion will appear radiographically. However, a carious lesion will not be identified radiographically until approximately 30-40% mineral loss has occurred and underestimates the extent of a lesion (Wenzel et al., 1990, Razmus, 1994, Shwartz et al., 1984). As a radiographic image provides a two-dimensional representation of a three-dimensional object, it can also be difficult to identify the exact geometric position of a carious lesion.

1.7.1 The detection of carious lesions using radiography

Radiographs are the most frequently used adjunct to visual examination in clinical practice for detecting carious lesions, however, great variability exists regarding its reported accuracy between studies (Bader et al., 2001). A recent systematic review and meta-analysis by Schwendicke *et al.* (2015) investigated radiographic caries detection. It found that clinically, radiographic detection has a generally low sensitivity for identifying all carious lesions (enamel and dentinal) associated with both occlusal surfaces (0.35), and proximal surfaces (0.24), however, its specificity is a lot higher (0.78 and 0.97 for occlusal and proximal surfaces respectively) (Schwendicke et al., 2015). Higher sensitivity values were identified when only carious lesions in dentine were assessed (0.56 and 0.36 for occlusal and proximal surfaces respectively), with the specificity values similar (0.95 and 0.94 for occlusal and proximal surfaces respectively), however, the highest sensitivity and specificity values were associated with the detection of cavitated proximal carious lesions (0.64 and 0.98 respectively) (Schwendicke et al., 2015). These sensitivity and specificity values are very similar to two previous systematic reviews (Bader et al., 2002, Gomez et al., 2013).

In conclusion, the most recent and comprehensive systematic review (Schwendicke et al., 2015) found radiographs to have high accuracy in detecting cavitated proximal carious lesions and well suited to detecting carious lesions in dentine which may present as ‘hidden’ lesions (Ricketts et al., 1997, Schwendicke et al., 2015), which agrees with another review (Bader et al., 2001). Also in agreement with Bader *et al.* (2001) there was little variation in the reported accuracy in radiographic detection of carious lesions between *in-vivo* and *in-vitro* studies (Schwendicke et al., 2015).

Fifteen years ago, in 2001, the National Institutes of Health (NIH) held a development conference and their consensus statement for the diagnosis and management of dental caries throughout life stated that ‘digitally acquired and post-processed images have great potential in the detection of non-cavitated caries and in the diagnosis of secondary caries’ but, that ‘developing digital imaging systems require robust laboratory and clinical evaluation’ (NIH, 2001). Since this statement, studies have investigated the use of digitally acquired and post-processed radiographic images for detecting and monitoring carious lesions, which have included the use of DSR, and will be discussed in the next section.

1.8 The detection and monitoring of carious lesions using DSR

There is considerable heterogeneity in the design of the small number of studies that have investigated the use of DSR for detecting and monitoring the progression of carious lesions over time which makes it difficult to carry out meaningful comparisons.

1.8.1 The detection of carious lesions using DSR at a single point in time

Three studies have investigated the accuracy of DSR for detecting carious lesions at a single point in time. These require contrast media such as stannous fluoride (Halse et al., 1990, Wenzel and Halse, 1992) and barium sulphate (Valizadeh et al., 2008) to increase the radiographic density of the lesion to enable it to be visualised after digital subtraction.

The use of DSR in combination with stannous fluoride results in an increase in the radiographic density of non-cavitated proximal carious lesions identified on the digital subtraction image (Halse et al., 1990) which is not seen when barium sulphate is used (Valizadeh et al., 2008). Although the relative increase seen in the radiographic density of carious lesions on digital subtraction images produced using stannous fluoride is lower in enamel than dentine, it does enable the detection of clinically present proximal white spot carious lesions that are not visible on digital radiographs (Halse et al., 1990). If a non-cavitated proximal carious lesion can be identified on a digital radiograph, the use of DSR in combination with stannous fluoride does not result in a statistically significant difference ($P>0.05$) in the measurement of the size of the lesion (Halse et al., 1990). However, if a cavitated proximal carious lesion can be identified on both a digital radiograph and digital subtraction image produced in combination with barium sulphate, the measurement of carious lesion depth is statistically significantly more accurate on the digital subtraction image (P=0.052) than digital radiograph (P<0.001) compared to the histologically validated carious lesion depth (Valizadeh et al., 2008).

The use of DSR in combination with stannous fluoride does not appear to improve the ability to detect non-cavitated occlusal carious lesions that extend into dentine compared to digital radiographs as more than 40% of teeth with such lesions were not detected by either method (Wenzel and Halse, 1992). These findings agree with those in a comparable sample of occlusal lesions using conventional radiographs (Wenzel and Fejerskov, 1992).

1.8.2 Detecting and monitoring the progression of carious lesions using DSR over time

A number of *in-vitro* and *in-vivo* studies have investigated DSR with and without contrast media for qualitatively and quantitatively detecting and monitoring the progression of proximal and occlusal carious lesions in enamel and dentine over time.

1.8.2.1 *In-vitro* studies

1.8.2.1.1 *In-vitro* studies involving carious lesions in enamel

DSR has been shown to be statistically significantly more accurate than pairwise comparison of radiographic images for detecting acid induced demineralisation within the proximal surfaces of enamel (Ferreira et al., 2006, Haiter-Neto et al., 2005). Haiter-Neto *et al.* (2005) identified no statistical significant difference (P>0.05) in accuracy between the use of logarithmically (AuROC curve = 0.98) and linear (AuROC curve = 0.97) contrast enhanced digital subtraction images, however, both were statistically significantly more accurate for detecting artificially created proximal lesions in enamel (P=0.0000) than pairwise comparison of conventional (AuROC curve = 0.90), CCD CygnusRay MPS (AuROC curve = 0.85), PSP DenOptix (AuROC curve = 0.91), PSP DIGORA (AuROC curve = 0.89) and digitised radiographic images (AuROC curve = 0.84). These findings were supported in a subsequent study by Ferreira *et al.* (2006), who demonstrated that logarithmically contrast enhanced digital subtraction images (AuROC curve = 0.98) were statistically significantly more accurate (P<0.05) than pairwise comparison of conventional (AuROC curve = 0.9), digitised (AuROC curve = 0.84) and digital radiographic images (AuROC curve = 0.91) for detecting artificially created proximal demineralisation lesions in enamel. Both studies revealed no statistically significant differences (P>0.05) in detection accuracy comparing digital subtraction images produced using digital radiographic images versus digitised radiographic images (Ferreira et al., 2006, Haiter-Neto et al., 2005).

However, these findings conflict with an earlier study where pairwise comparison of conventional radiographs (AuROC curve = 0.7 and 0.9) was statistically significantly more accurate (P<0.05) than digital subtraction images with linear contrast enhancement (AuROC curve = 0.66 and 0.85) in detecting proximal lesions created mechanically, to represent 5% and 10% enamel mineral loss respectively (Halse et al., 1994). The conflicting findings are likely to be due to the mechanically prepared lesions resulting in well-defined radiographic lesions with sharp borders giving a higher contrast to air compared to the lesions created using demineralising solution in the other two studies (Ferreira et al., 2006, Haiter-Neto et al., 2005). Demineralising solution is considered to be more representative of clinical lesions than mechanically prepared ones, so the Ferreira *et al.* (2006) and Haiter-Neto *et al.* (2005) studies are more likely to represent accuracy in the clinical situation.

1.8.2.1.2 *In-vitro* studies involving carious lesions in dentine

DSR can detect demineralisation associated with naturally occurring (Maggio et al., 1990) and artificially created (Minah, 1998) carious lesions over time. Maggio *et al.* (1990) used traditional subtraction radiography techniques and observed that over an eight week period, digital subtraction images showed a statistically significant increase (P=0.011) in the detection of a radiolucency at the deepest extent of naturally occurring carious lesions in dentine in teeth stored in a saliva buffer with 5% glucose or 5% sucrose compared to teeth stored in a saliva buffer alone. Interestingly, an increase in radiopacity at the deepest extent of the carious lesions stored in the saliva buffer alone was identified on a number of the digital subtraction images which likely represents detection of remineralisation of the advancing front of the carious lesion due to deposition of calcium salts. The type of teeth used or the location of the carious lesions in their study was not specified.

Minah (1998) investigated quantitative methods in combination with DSR for assessing the extent of demineralisation of artificially created occlusal cavities in primary teeth incubated in cariogenic cultures over 45 days. DSR values for the carious lesions were calculated by multiplying the average grey-scale value of the ROI around the carious lesion by the area of the ROI which corresponded to mineral loss, which is similar to a Computer-Assisted Densitometric Image Analysis (CADIA) value (Schmidlin et al., 2002). The digital subtraction values for the ROI reduced over time, for all teeth, representing the ability of DSR to detect demineralisation and lesion progression over time (Minah, 1998).

Ricketts *et al.* (2007) investigated the accuracy and reproducibility of DSR for detecting demineralisation in dentine (not reported by Maggio *et al.* (1990) or Minah (1998)) in addition to carrying out a pairwise comparison of digital radiographs. Demineralising solution was placed in occlusal cavities extending into dentine over 24 hours and at baseline and after 3 and 6 hours, there was no statistically significant difference (P>0.05) in the accuracy for detecting occlusal demineralisation between the digital subtraction images (AuROC curve = 0.58, 0.64 and 0.71 respectively) and pairwise comparison of the digital images (AuROC curve = 0.5, 0.55 and 0.58 respectively). However, after 12, 18 and 24 hours, DSR (AuROC curve = 0.95, 0.98 and 0.96 respectively) was statistically significantly (P<0.01) more accurate at detecting occlusal demineralisation than pairwise comparison of digital radiographs (AuROC curve = 0.61, 0.65 and 0.76 respectively). This agrees with the findings of the Haiter-Neto *et al.* (2005) and Ferreira *et al.* (2006) studies regarding the detection of acid induced demineralisation within the proximal surfaces of enamel. Ricketts *et al.* (2007) found no statistically significant differences (P>0.05) in mean intra-examiner reproducibility over 24 hours, or mean inter-examiner reproducibility at baseline or after 3 hours between pairwise comparison of digital radiographs and digital subtraction images. However, after 6 hours or more there was a statistically significant difference (P<0.05) in mean inter-examiner reproducibility favouring DSR (Ricketts et al., 2007).

The majority of the *in-vitro* studies discussed above provide evidence to support the statement that DSR is more accurate than pairwise comparison of digital radiographs for detecting carious lesions in enamel and dentine.

1.8.2.1.3 *In-vitro* studies involving recurrent carious lesions

Two *in-vitro* studies have assessed DSR for detecting recurrent carious lesions, however, due to differences in study design the results cannot be directly compared to one another (Eberhard et al., 2000, Nummikoski et al., 1992). Nummikoski *et al.* (1992) assessed the accuracy and reproducibility of DSR compared to conventional radiography for detecting mechanically created proximal defects underneath two different restorative materials. They demonstrated that DSR was statistically significantly more accurate than using conventional radiography in detection of recurrent carious lesions under Valux (3M, St Paul, MN, USA) ($P<0.0001$) and Fulfil composites (L.D. Caulk, Milford, DE, USA) ($P=0.0004$). Conventional radiography may have been biased against in this study as only the post-defect radiographic film was provided to examiners and, the pre-defect radiographic film was not available for pairwise comparison. Although Valux has an attenuation coefficient considerably lower than enamel and Fulfil has an attenuation coefficient higher than enamel, this did not have a statistically significant effect on the accuracy to detect recurrent carious lesions using DSR (AuROC curve = 0.931 and 0.948 respectively), but did have a statistically significant effect when using conventional radiography (AuROC curve = 0.577 and 0.892 respectively). Nummikoski *et al.* (1992) also found that DSR had a statistically significantly higher ($P=0.0258$) inter-examiner reproducibility (kappa value = 0.671) compared to conventional radiography (kappa value = 0.541), however no statistically significant difference ($P=0.2950$) in intra-examiner reproducibility was identified comparing DSR (kappa value = 0.760) with conventional radiography (kappa value = 0.656), similar to Ricketts *et al.* (2007) for occlusal caries.

Eberhard *et al.* (2000) investigated quantitative analysis of grey-scale values using digital subtraction images to detect acid induced demineralisation over 42 days on the proximal surfaces of unrestored non-carious teeth and teeth restored with glass ionomer restorative material using a modified tunnel preparation technique. When the crown of the whole tooth was analysed, there was no statistically significant difference in mean grey-scale value over 7-42 days, however, a statistically significant difference was detected when the proximal surface and maximum size of the proximal lesion were analysed in isolation. The reduction in mean grey-scale value was less for the lesions associated with the proximal surfaces that had been restored with glass ionomer modified tunnel preparations, compared to the unrestored non-carious teeth, however, the difference was not statistically significant (P>0.05).

The findings of this study together with those of Minah (1998) suggest that the quantitative analysis of mean grey-scale values from digital subtraction radiographic images could be used to monitor carious lesions, but that only the lesion should be analysed in isolation, rather than the whole tooth (Eberhard et al., 2000). *In-vivo* studies investigating quantitative analysis of mean grey-scale values for detecting and monitoring carious lesions are discussed later.

1.8.2.1.4 *In-vitro* studies involving the use of contrast enhancement and DSR

Two studies have compared the accuracy of contrast enhanced and standard digital subtraction images against each other for detecting carious lesions (Grondahl et al., 1982, Versteeg and van der Stelt, 1995). Grondahl *et al.* (1982) used computer software to artificially create caries like proximal lesions in enamel on digitised radiographs of clinically and radiographically sound teeth, and also adjusted the densitometric parameters to simulate underexposure. Logarithmically enhanced and standard digital subtraction images were produced and viewed by examiners who recorded their certainty as to whether or not a carious lesion was present. Significantly higher accuracies were obtained when logarithmically contrast enhanced digital subtraction images were viewed compared to unenhanced digital subtraction images using underexposed radiographs, however, for normally exposed radiographs, there was no statistical difference. Versteeg and van der Stelt (1995) also compared the accuracy of logarithmically enhanced digital subtraction images with unenhanced digital subtraction images for detecting computer generated lesions on a plain texture, bone and enamel. Two groups of examiners (one containing dentists and one containing final year dental students) viewed the images and recorded their certainty as to whether or not a carious lesion was present. The accuracy for detecting the computer generated carious lesions in enamel was statistically significantly higher (P<0.05) using the logarithmically contrast enhanced digital subtraction images compared to unenhanced digital subtraction images in the student group, but not in the group of dentists, or when the accuracy of both groups was compared as one. They also concluded, in agreement with Halse *et al.* (1994), that structural noise has a significant effect on the accuracy of detecting lesions using digital subtraction images.

Contrast enhanced digital subtraction images therefore appear to improve the accuracy of detecting carious lesions over standard digital subtraction images under a number of specific conditions including, when the digital radiographs being used to produce the image are underexposed (Grondahl et al., 1982); or the digital subtraction images are being viewed by inexperienced examiners (Versteeg and van der Stelt, 1995).

In addition, although accuracy was not improved between logarithmically and linear contrast enhanced digital subtraction images for detecting artificially created proximal demineralisation lesions in enamel, logarithmically contrast enhanced digital subtraction images did reduce variation in accuracy (Haiter-Neto et al., 2005).

1.8.2.1.5 Conclusions and issues with the *in-vitro* studies

The majority of the *in-vitro* studies discussed above demonstrate that DSR can detect and monitor carious lesions over time, and that it is at least as reproducible and significantly more accurate than the digital or conventional radiographic methods currently used in clinical practice. The accuracy and reproducibility achieved in the *in-vitro* studies for DSR, and to some extent digital and conventional radiography is however likely to be higher than that achieved *in-vivo* in the clinical setting for a number of reasons including:

1. it is more difficult to standardise X-ray projection geometry clinically, even when using customised devices;
2. variations in densitometric parameters are also harder to control clinically, even if the same exposure parameters are selected and the same X-ray unit, radiographic film or sensor and X-ray projection geometry are used. Human factors such as soft tissue attenuation, the movement of teeth, the presence of restorations and toothwear will all lead to variations in densitometric parameters in the same patient; and
3. in the clinical situation, teeth are not viewed in isolation of other anatomic structures unlike *in-vitro*, where they are viewed as a single unit.

These factors are all likely to contribute to a reduction in the quality of the *in-vivo* digital subtraction image. Additional differences that might contribute to changes in accuracy of diagnostics between *in-vitro* and *in-vivo* studies are:

1. differences in study designs as in *in-vitro* laboratory studies, examiners are often aware in which surface or location of the tooth they would be expected to potentially detect a carious lesion, reducing the complexity of decision making often encountered in the *in-vivo* clinical setting; and
2. the nature of the carious lesion itself as in *in-vitro* studies, existing natural carious lesions are either identified or created artificially, and even if an existing natural carious lesion is identified, it is difficult to mimic artificially progression of the lesion; in the *in-vivo* clinical setting this involves a complex dynamic process which involves both demineralisation and remineralisation over a significant period of time which is often longer than the period that studies are carried out.

1.8.2.2 *In-vivo* studies

In-vivo clinical studies have investigated the accuracy and reproducibility of subtraction radiography for qualitatively and quantitatively detecting and monitoring carious lesions in enamel and dentine on proximal and occlusal tooth surfaces. This includes studies that have investigated a variety of techniques ranging from non-invasive approaches involving topical fluoride, sealants and resin infiltration to more invasive techniques incorporating stepwise excavation. For ethical reasons, histological validation is rarely possible, so the accuracy and reproducibility of subtraction radiography has often been validated against other detection methods, in particular the use of conventional radiographic techniques. Due to the low number of studies and the differences in their study design, it is difficult to make meaningful comparisons between the studies that have been published, however, they will be discussed below.

1.8.2.2.1 *In-vivo* studies involving quantitative analysis

Five *in-vivo* studies have investigated DSR for assessing the progression of carious lesions over time using quantitative measurement of mean grey-scale values on digital subtraction images, however, no comparisons were made with any other detection methods and the mean grey-scale values were validated against qualitative assessment of lesion change on the same digital subtraction image (Alves et al., 2009, Carneiro et al., 2009, Maltz et al., 2002, Maltz et al., 2007, Oliveira et al., 2006). All five studies produced digital subtraction images from digitised conventional bitewing radiographs following density normalisation, and limited quantitative measurement to a specific ROI involving part of, or the whole carious lesion in its entirety, rather than the tooth as a whole as recommended by Eberhard *et al.* (2000).

Carneiro *et al.* (2009) recruited patients with conventional radiographic evidence of proximal carious lesions extending into enamel which were subsequently managed with oral hygiene instruction, dietary interventions and remineralisation therapy on a weekly basis over 8 weeks which involved carrying out dental prophylaxis and conditioning of the lesion using 37% phosphoric acid gel and 0.05 M aluminium nitrate prior to the topical application of 1.23% acidulous phosphate fluoride gel. Pre- and post-management radiographs were taken using the same X-ray tube vertical angulation and commercially available bitewing film holders with customised silicone bite blocks to standardise X-ray projection geometry. Qualitative analysis revealed 10 carious lesions were judged to have demineralised, 34 remained unchanged and 17 remineralised and the respective mean grey-scale values of the carious lesions in each group were 112.10, 127.29 and 137.47 which were significantly different ($P<0.05$) from one another.

Four *in-vivo* studies reported the results of the same cohort of patients who underwent stepwise excavation to manage deep carious lesions in dentine over time; results at 6-7 months (Maltz et al., 2002), 14-18 months (Oliveira et al., 2006), 36-45 months (Maltz et al., 2007) and 10 years (Alves et al., 2009). Radiographs were taken at baseline following first excavation and placement of the temporary restoration and after each time period before re-entering, using a commercially available bitewing film holder with customised self-cured acrylic resin bite block to standardise X-ray projection geometry. Interestingly, after 10-years due to changes in tooth position and distortion of the self-cure acrylic resin the customised bite blocks could not be used, so standard bite blocks were used instead. Statistically significant differences ($P<0.05$) between the mean grey-scale value of the radiolucent zones immediately below the restorations compared to control zones in the adjacent dentine were identified at each time period, with the radiolucent zone having a higher mean grey-scale value compared to the control zones. Intra-examiner reproducibility of the quantitative measurement of the radiolucent zones of the carious lesions carried out using the digital subtraction images found increasing intraclass correlation coefficient values over time; 0.69 (6-7 months), 0.83 (14-18 months), 0.82 (36-45 months) and 0.99 (10 years).

The five *in-vivo* studies (Alves et al., 2009, Carneiro et al., 2009, Maltz et al., 2002, Maltz et al., 2007, Oliveira et al., 2006) suggest that quantitative measurement of the mean grey-scale value of part of, or, a whole carious lesion confined to enamel or extending into dentine using DSR enables monitoring of further demineralisation, remineralisation or arrest over time, and that the intra-examiner reproducibility for carrying out quantitative measurements is high. However, none of the studies reported inter-examiner reproducibility and the sample sizes were relatively small.

1.8.2.2.2 *In-vivo* studies involving qualitative analysis

Four *in-vivo* studies have investigated and compared the qualitative analysis of DSR with other conventional radiographic methods for assessing the progression of carious lesions in both enamel and dentine over time (Martignon et al., 2006, Martignon et al., 2012, Paris et al., 2010, Wenzel et al., 2000). Three of them (Martignon et al., 2006, Martignon et al., 2012, Paris et al., 2010) also compared the effect of various carious lesion management interventions including preventative strategies (incorporating oral hygiene instruction, dietary interventions and the application of topical fluoride), the placement of sealants and resin infiltration. A summary of the individual study designs and their materials and methods is presented in Table 3. The progression of carious lesions was not validated histologically in any of the four studies, however, its detection on digital subtraction images was compared to its detection using other conventional radiographic methods for the different carious lesion management interventions used in the studies carried out by Martignon *et al.*, (2006 and 2012) and Paris *et al.* (2010).

DSR detected that a higher proportion of carious lesions had progressed over time compared to conventional independent (Martignon et al., 2006), conventional pairwise (Martignon et al., 2006, Martignon et al., 2012) and digitised pairwise (Paris et al., 2010) comparison of radiographs (Table 4). Unfortunately, as these three studies were primarily concerned with comparing different carious lesion management interventions, no statistical analysis was carried out to assess for any significant differences between the radiographic methods used for detecting carious lesion progression.

DSR was also shown to have higher intra-examiner reproducibility for assessing carious lesion progression compared to conventional independent (Martignon et al., 2006) and conventional pairwise comparison of radiographs (Martignon et al., 2006, Martignon et al., 2012, Wenzel et al., 2000) (Table 5). Inter-examiner reproducibility for assessing carious lesion progression was however shown to be higher than digitised independent and pairwise comparison of digitised radiographs in the study by Paris *et al.* (2010), but lower than pairwise comparison of conventional radiographs in the study by Wenzel *et al.* (2000), however this difference was not statistically significant (Table 5).

Table 3. A summary of the study designs and materials and methods for the four *in-vivo* studies that investigated the qualitative analysis of DSR

Study	**Wenzel *et al.* (2000)**	**Martignon *et al.* (2006)**	**Paris *et al.* (2010)**	**Martignon *et al.* (2012)**
Number of participants	49	82	22	22
Power calculation	No	Yes	Yes	Yes
Tooth surface	Any surface	Proximal	Proximal	Proximal
Lesion type	Enamel and dentine	Enamel (outer 1/2, inner 1/2) and dentine (outer 1/3)	Enamel (inner 1/2) and dentine (outer 1/3)	At ADJ and dentine (outer 1/3)
Time period	1-2 years	18 months	18 months	1 year
Treatment groups	-	Split mouth	Split mouth	Split mouth
Management interventions	-	Prevention Prevention and sealing	Prevention and resin infiltration Prevention and placebo	Prevention and resin infiltration Prevention and sealing Prevention and placebo
Randomised management allocation	-	Yes	Yes	Yes
Standardised X-ray projection geometry	Yes	Yes	Yes	Yes
Density normalisation	Not mentioned	Yes	Not mentioned	Not mentioned
DSR compared to	Conventional pairwise comparison	Conventional pairwise comparison Conventional independent	Digitised pairwise comparison	Conventional pairwise comparison
Number of examiners	7	1	2	1
Assessment of lesion change	Qualitative	Qualitative	Qualitative	Qualitative

Table 4. A summary of the results of lesion progression (as indicated by %) for the four *in-vivo* studies that investigated the qualitative analysis of DSR

Study	Wenzel *et al.* (2000)**	Martignon *et al.* (2006)	Paris *et al.* (2010)	Martignon *et al.* (2012)
Lesion progression assessed by DSR		(* denotes stat. sig. diff. P<0.05 with McNemar test)	(* denotes stat. sig. diff. P<0.05 with McNemar test)	(* denotes stat. sig. diff. P<0.05 with McNemar test)
Prevention (P)		84.5%*	37%*	63%* (P vs SP and P vs RP)
Sealing and prevention (SP)		43.4%*		42%* (P vs SP)
Resin infiltration and prevention (RP)			7%*	26%* (P vs RP)
Lesion progression assessed by conventional independent		(* denotes stat. sig. diff. P<0.05 with McNemar test)		
Prevention (P)		26.40%		
Sealing and prevention (SP)		9.7%		
Lesion progression assessed by conventional pairwise		(* denotes stat. sig. diff. P<0.05 with McNemar test)		(* denotes stat. sig. diff. P<0.05 with McNemar test)
Prevention (P)		47.2%*		47%* (P vs RP)
Sealing and prevention (SP)		22.2%*		29%
Resin infiltration and prevention (RP)				16%* (P vs RP)
Lesion progression assessed by digitised pairwise			(* denotes stat. sig. diff. P<0.05 with McNemar test)	
Prevention (P)			22%	
Resin infiltration and prevention (RP)			4%	

**This study did not report lesion progression but for continuity has been included in Table 4

Table 5. A summary of the intra- and inter-examiner reproducibility for the four *in-vivo* studies that investigated the qualitative analysis of DSR

Study	Wenzel *et al.* (2000)	Martignon *et al.* (2006)	Paris *et al.* (2010)	Martignon *et al.* (2012)
Intra-examiner reproducibility	Kappa (* denotes stat. sig. diff. P<0.05 using Wilcoxons non-parametric test for paired data)	Kappa (% Agreement) (Stat. sig. diff. not tested for)	Kappa Ranged from 0.510 - 0.894 (Stat. sig. diff. not tested for)	Kappa (Stat. sig. diff. not tested for)
DSR	0.875*	0.87 (92%)		0.78
Conventional independent		0.84 (96%)		
Conventional pairwise	0.758*	0.44 (68%)		0.74
Inter-examiner reproducibility	Kappa (* denotes stat. sig. diff. P<0.05 using Wilcoxons non-parametric test for paired data)		Kappa (Stat. sig. diff. not tested for)	
DSR	0.678		0.809	
Digitised independent			0.585	
Conventional pairwise	0.701			
Digitised pairwise			0.674	

1.9 Conclusions

With the change in philosophy in the management of carious lesions towards preventative, micro-invasive and restorative techniques that promote the arrest, and remineralisation of the lesion, there is a need to develop new methods of monitoring lesion behaviour over time. If the carious biomass has been sealed within the tooth, using either a sealant, resin infiltration or a restoration, radiography is the only method available at present to monitor the lesion over time. The use of digital subtraction images has been demonstrated to be more accurate and reproducible for detecting and monitoring carious lesion progression over time compared to pairwise comparison of conventional or digital radiographs.

It is recognised that the use of a reproducible X-ray projection geometry improves the quality of the production of a digital subtraction image. Although digital subtraction software can compensate for subject-film errors, no studies to date have investigated the effect that variations in X-ray source subject projection geometry have on the accuracy and reproducibility for detecting carious lesion progression. Neither have any studies investigated the use of a grading system for assessing observed changes in the proximal relationships of teeth on paired digital radiographs that have been taken with variations in X-ray source subject projection geometry, and evaluated their suitability to undergo digital subtraction to detect carious lesion progression.

It is essential to investigate these areas further to provide evidence to support rational decision making around whether DSR could be used in the clinical setting to accurately and reliably monitor carious lesion progression, as the X-ray projection geometry cannot be standardised completely. This information will also allow identification of gaps in knowledge and areas for future development of techniques to improve diagnosis of carious lesion behaviour.

2 Aims and objectives

2.1 Research question

Does alteration of X-ray source subject projection geometry have an impact on the accuracy and reproducibility of DSR for detecting demineralisation in artificially created occlusal cavities?

2.2 Aim 1

Part 1 of this research project involves *in-vitro* assessment of the accuracy and reproducibility of DSR for detecting demineralisation in occlusal cavities using digital radiographs taken with variations in X-ray source subject projection geometry after varying time periods of demineralisation.

2.2.1 Objectives

1. Evaluate the effect on accuracy for detecting demineralisation in occlusal cavities, that variations in the horizontal (by 7 and 15 degrees) and vertical (by 10 and 15 degrees) X-ray source subject projection geometry of digital radiographs used to produce digital subtraction images have, when compared to the use of digital radiographs with a reproducible 0 degree X-ray projection geometry;
2. Determine whether there is any significant difference in the accuracy of DSR for detecting demineralisation in occlusal cavities using digital radiographs that have had a mesial, compared to a distal, 7 and 15 degree horizontal angulation variation in X-ray source subject projection geometry;
3. Determine whether there is any significant difference in the accuracy of DSR for detecting demineralisation in occlusal cavities using digital radiographs that have had a positive upward, compared to a negative downward, 10 and 15 degree vertical angulation variation in X-ray source subject projection geometry;
4. Evaluate the effect that variations in the horizontal (by 7 and 15 degrees) and vertical (by 10 and 15 degrees) X-ray source subject projection geometry of digital radiographs used to produce digital

subtraction images have on its intra-examiner reproducibility for detecting demineralisation in occlusal cavities; and

5. Evaluate the effect that variations in the horizontal (by 7 and 15 degrees) and vertical (by 10 and 15 degrees) X-ray source subject projection geometry of digital radiographs used to produce digital subtraction images have on its inter-examiner reproducibility for detecting demineralisation in occlusal cavities.

2.3 Aim 2

In light of the results arising from Aim 1 of this research project, which identified that the accuracy of DSR for detecting demineralisation in occlusal cavities decreased when digital radiographs were taken with increasing horizontal variations in X-ray source subject projection geometry, Part 2 of this research project investigated the discriminatory ability and evaluated the reproducibility of a grading system for assessing observed changes in the proximal relationships of teeth on paired digital radiographs that had been taken with horizontal variations in X-ray source subject projection geometry.

2.3.1 Objectives

1. Assess the median differences in scores for the proximal relationships of teeth on paired digital radiographs that have been taken with 7 and 15 degree horizontal angulation variations in X-ray source subject projection geometry between them;
2. Assess the intra-examiner reproducibility of a grading system for assessing the observed changes in the proximal relationships of teeth on paired digital radiographs that have been taken with 7 and 15 degree horizontal angulation variations in X-ray source subject projection geometry between them; and
3. Assess the inter-examiner reproducibility of a grading system for assessing the observed changes in the proximal relationships of teeth on paired digital radiographs that have been taken with 7 and 15 degree horizontal angulation variations in X-ray source subject projection geometry between them.

3 Materials and methods

Part 1: *In-vitro* the accuracy and reproducibility of DSR for detecting demineralisation in occlusal cavities using digital radiographs taken with variations in X-ray source subject projection geometry after varying time periods of demineralisation

3.1 Introduction

One-hundred and sixty digital radiographs were obtained to achieve Aim 1 of this research project. These included 100 pre- and 60 post-demineralisation digital radiographs. Eighty of these digital radiographs (20 pre- and 60 post-demineralisation) taken with a reproducible X-ray projection geometry were used in the *in-vitro* study by Ricketts *et al.* (2007) which investigated the accuracy and reproducibility of conventional radiographic assessment and DSR for detecting occlusal demineralisation. The additional 80 pre-demineralisation digital radiographs which were not used in the *in-vitro* study by Ricketts *et al.* (2007) were taken with variations in X-ray source subject projection geometry and have not been used to date. Section 3.2 describes how the 160 digital radiographs were produced prior to me obtaining them for use in this research project, and therefore, part of it details the materials and methods already discussed in the *in-vitro* study published by Ricketts *et al.* (2007). Section 3.3 describes how the 160 digital radiographs that were obtained were used in this research project. Figure 5 shows a flowchart summarising the materials and methods for this part of the research project.

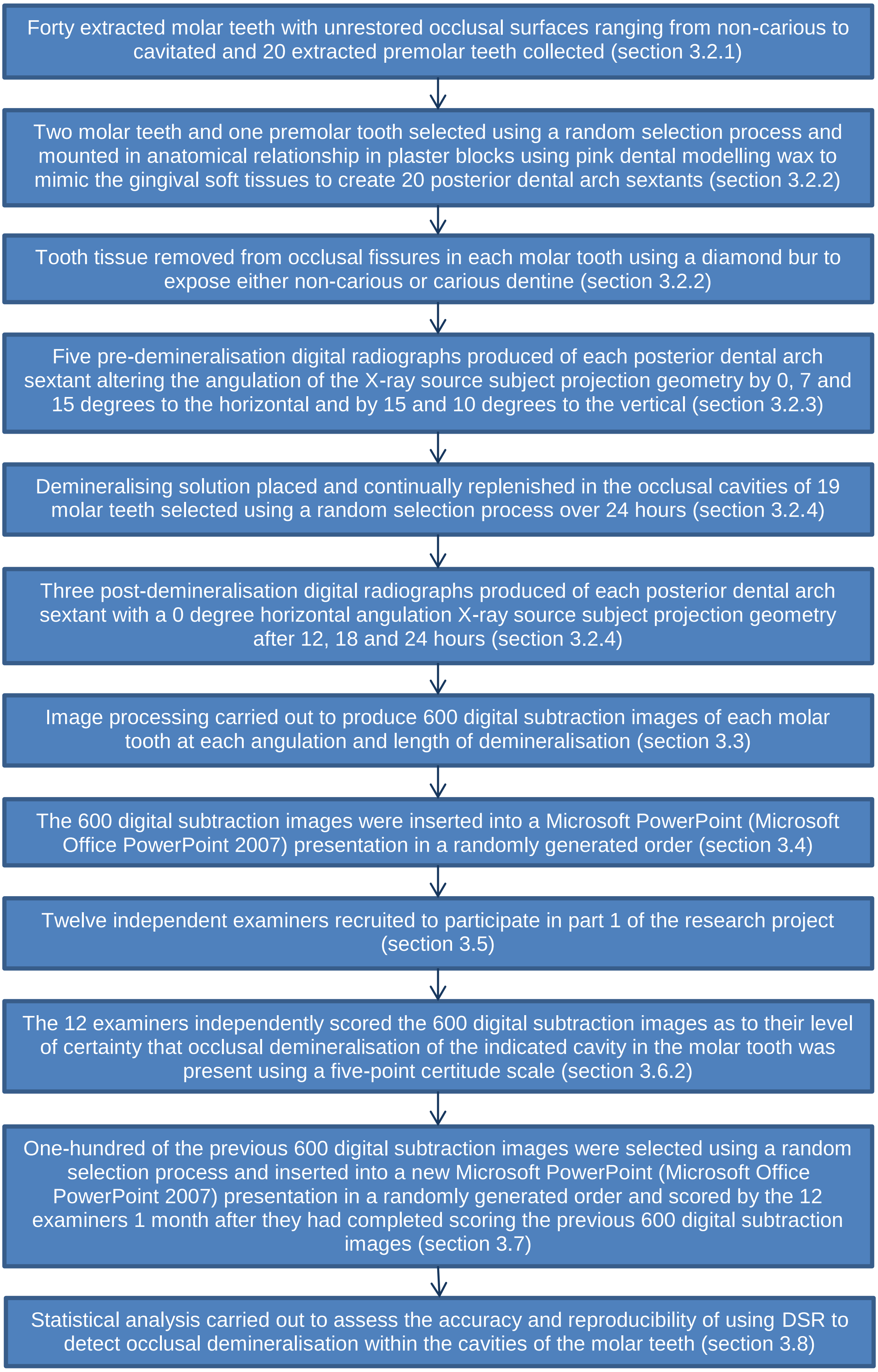


Figure 5. Flowchart summarising the materials and methods for Part 1 of this research project

3.2 Production of digital radiographs obtained for use in this research project

3.2.1 Collection of extracted teeth

Forty extracted molar teeth with unrestored occlusal surfaces ranging from non-carious to cavitated and 20 premolar teeth were selected from a bank of extracted teeth retained at Dundee Dental Hospital and School for teaching and research purposes. The teeth were cleaned and stored in saline containing thymol crystals to inhibit bacterial growth.

3.2.1.1 Ethical approval

The teeth were collected prior to the implementation of the Human Tissue (Scotland) Act 2006 and used in accordance with accepted standards at the time.

3.2.2 Set up of extracted teeth and preparation of occlusal cavities

Two molar teeth and one premolar tooth were selected using a random selection process to create twenty posterior dental arch sextants. The extracted teeth were mounted in anatomical relationship in plaster blocks reaching up to 3-4mm short of the ACJ. Pink dental modelling wax was placed over the plaster up to the ACJ and moulded to mimic the gingival soft tissues (Figure 6).

Figure 6. Posterior dental arch sextant

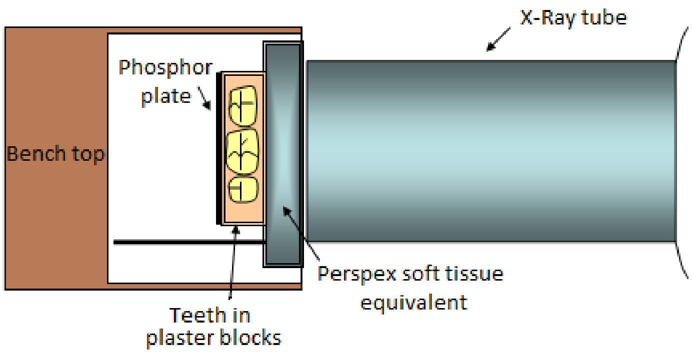
Five notional heads were created each comprising an upper right, upper left, lower left and lower right posterior dental arch sextant. A numerical code was applied to each of the forty molar teeth and used as a tooth identifier. The plaster blocks were trimmed to allow close approximation of PSP plates to the palatal/lingual surfaces of the teeth. This also allowed the PSP plates to be aligned parallel to the line of the sextant. Enamel was removed from the occlusal fissures in each molar tooth using a diamond bur to expose either the non-carious or carious dentine below. In the non-carious teeth the occlusal cavities were cut 2-3mm wide in the bucco-lingual direction. In the carious teeth, the enamel was removed to expose the carious dentine underneath (Figure 7).

Figure 7. Occlusal cavities cut in the molar teeth

3.2.3 Production of pre-demineralisation digital radiographs

Pre-demineralisation digital radiographs were obtained using an alignment system which allowed control over the X-ray projection geometry. A laminated board was placed on a horizontally level dental surgery cabinet. Two adjacent parallel rectangles were marked to enable accurate positioning of the plaster block containing the teeth and a 10mm thick perspex block which was used as a soft tissue equivalent. PSP plates (DenOptix™ PSP digital imaging system), within their light protective sleeves, were attached to the palatal/lingual surface of the plaster blocks, parallel to the teeth. The plaster blocks were positioned with the buccal surface of the teeth next to the perspex block, facing the X-ray tube (Figure 8).

A



B

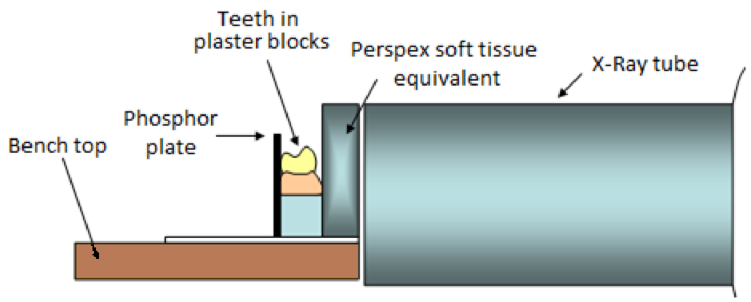


Figure 8. Aerial (A) and horizontal (B) view of the set-up for production of the digital radiographs

Digital radiographs were taken using a Gendex 765DC X-ray source (65kV, 7 mA, exposure time 0.16s, source-to-detector distance 250mm). The PSP plates were read using a DenOptix scanner and VixWin software and the digital images stored as .tif images (dimensions 865x576 pixels, 300 dpi, bit depth 24, compression LZW, resolution unit 2).

Five pre-demineralisation digital radiographs were taken of each posterior dental arch sextant. The 0 degree angulation pre-demineralisation digital radiograph was taken with the vertical angulation of the long axis of the X-ray tube parallel to the horizontal plane (Figure 9 D) and the end of the X-ray tube perpendicular to and flat against the surface of the perspex block (Figure 9 A). The X-ray beam passed tangentially through the inter-proximal space and/or contact points of the molar and premolar teeth (Figure 8). The perspex block, plaster block and PSP plate were positioned parallel to each other (Figure 8). Four further pre-demineralisation digital radiographs were taken following alteration of the X-ray source subject projection geometry compared to the 0 degree angulation pre-demineralisation digital radiograph. The horizontal angulation of the end of the X-ray tube was rotated by 7 degrees (Figure 9 B) and 15 degrees (Figure 9 C) anti-clockwise (as viewed from the aerial perspective), and the vertical angulation of the long axis of the X-ray tube altered by 15 degrees (upward direction) (Figure 10 A) and 10 degrees (downward direction) (Figure 10 B). The perspex block, plaster block and PSP plates' geometric relationship remained constant, therefore, changes were only made to the X-ray source subject projection geometry. Five pre-demineralisation digital radiographs were therefore produced for each posterior arch dental sextant with varying X-ray source subject projection geometry.

Each of the 20 posterior dental arch sextants was positioned with the buccal surface of the teeth towards the X-ray beam and on the base of the plaster block. Alteration of the horizontal angulation of the long axis of the end of the X-ray tube by 7 degrees and 15 degrees anti-clockwise, to a line drawn perpendicular from the surface of the perspex block as viewed from the aerial perspective for the production of the 7 degree and 15 degree horizontal angulation pre-demineralisation digital radiographs, resulted in a distal shift of the X-ray beam for the lower left and upper right posterior dental arch sextants and mesial shift of the X-ray beam for the lower right and upper left posterior dental arch sextants. Alteration of the vertical angulation of the long axis of the X-ray tube by 15 degrees (upward direction), for the production of the 15 degree vertical angulation pre-demineralisation digital radiograph, resulted in a 15 degree upward direction shift of the X-ray beam for the lower posterior dental arch sextants and a 15 degree downward direction shift of the X-ray beam for the upper posterior dental arch sextants. Alteration of the vertical angulation of the long axis of the X-ray tube by 10 degrees (downward direction) for the production of the 10 degree vertical angulation pre-demineralisation digital radiograph resulted in a 10 degree downward direction shift of the X-ray beam for the lower posterior dental arch sextants and a 10 degree upward direction shift of the X-ray beam for the upper posterior dental arch sextants.

A

B

C

D

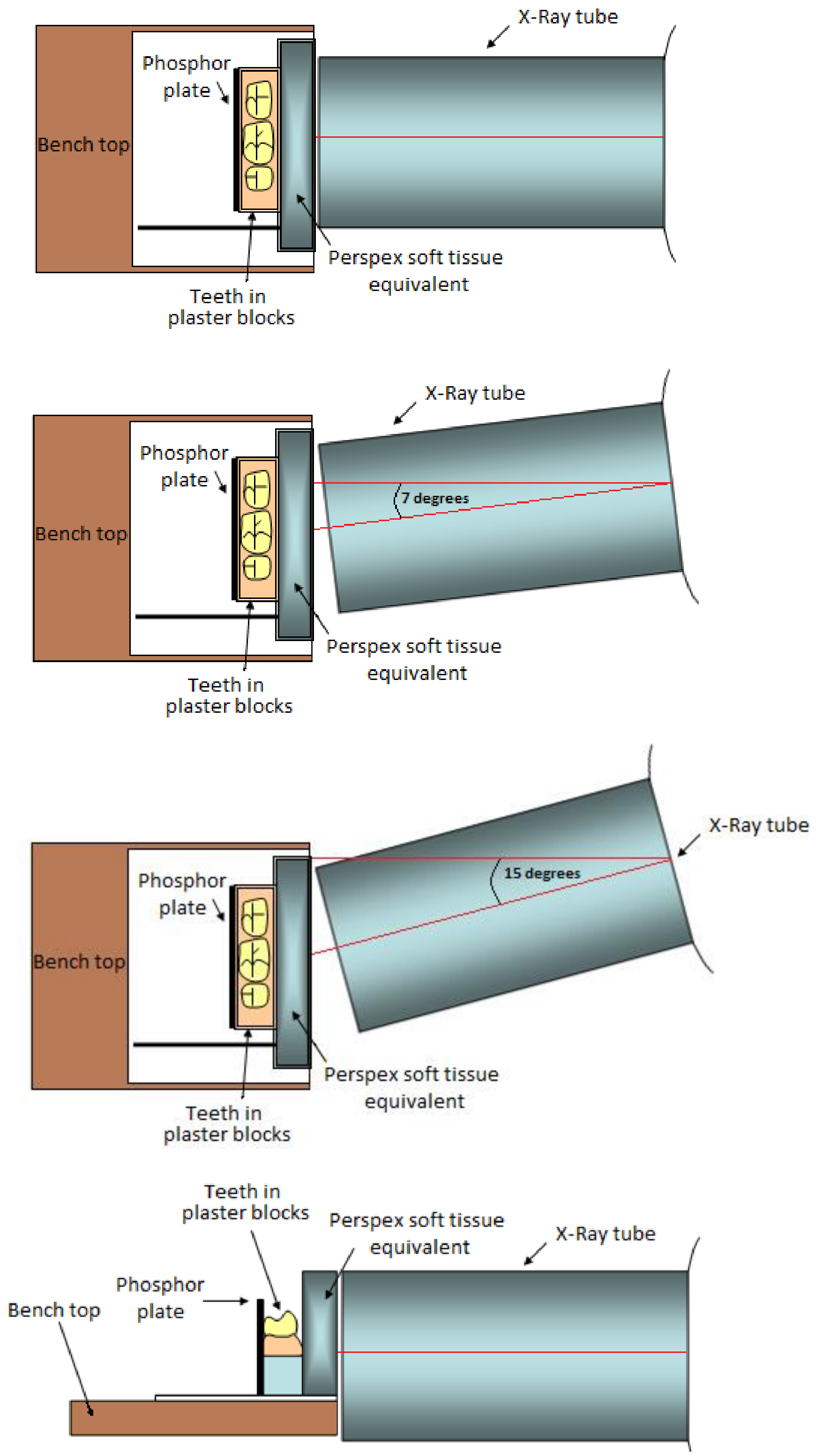


Figure 9. Aerial (A,B,C) and horizontal (D) views of the X-ray projection geometry set-up for production of the 0 degree angulation (A&D), 7 degree horizontal angulation (B&D) and 15 degree horizontal angulation (C&D) pre-demineralisation digital radiographs

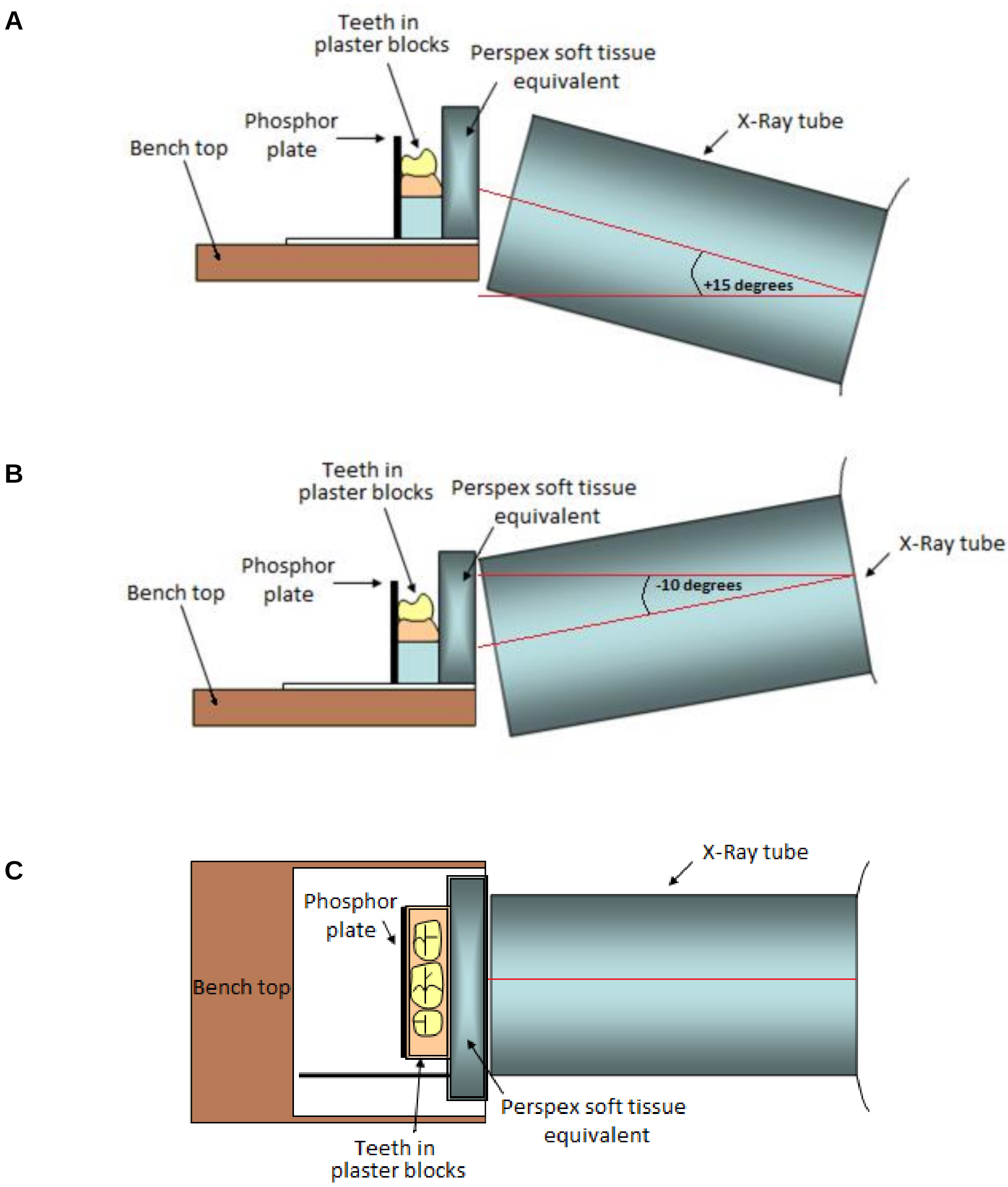


Figure 10. Horizontal (A,B) and aerial (C) views of the X-ray projection geometry set-up for production of the 15 degree vertical angulation (A&C) and 10 degree vertical angulation (B&C) pre-demineralisation digital radiographs

3.2.4 Demineralisation of occlusal cavities and production of post-demineralisation digital radiographs

Nineteen of the 40 molar teeth were randomly selected for demineralisation. A demineralising solution (ph=1; Surgipath Decalcifier II) was dropped with a pipette into the occlusal cavities in these 19 molar teeth and continually replenished over a 24-hour period. The demineralising solution was rinsed out of the occlusal cavities after 12, 18 and 24 hours and post-demineralisation digital radiographs taken of all 20 posterior dental arch sextants at these time intervals. Zero degree post-demineralisation digital radiographs were taken using the same X-ray projection geometry that was used to take the 0 degree angulation pre-demineralisation digital radiographs (Figure 9 A&D) that is, with the vertical angulation of the long axis of the X-ray tube parallel to the horizontal plane and the end of the X-ray tube perpendicular to and flat against the surface of the perspex block.

The following digital radiographs were therefore obtained for each of the 20 posterior dental arch sextants:

- Five pre-demineralisation digital radiographs taken with 0 degree, 7 degree horizontal, 15 degree horizontal, 10 degree vertical and 15 degree vertical angulation changes in X-ray source subject projection geometry; and
- Three 0 degree angulation post-demineralisation digital radiographs taken after 12, 18 and 24 hours demineralisation.

The resulting 160 digital radiographs were saved as .tif files with file names to enable identification of:

1. Which posterior dental arch sextant was used;
2. Which X-ray source subject projection geometry was used; and
3. If the digital radiograph was taken pre-demineralisation or after 12, 18 or 24 hours post-demineralisation.

3.3 Production of digital subtraction images

3.3.1 Investigator and examiner blinding

The resulting 160 digital radiographs were obtained by the principle researcher Samuel Rollings (SR), however, the identity of the 19 demineralised molar teeth was only made available once the digital subtraction images had been produced and scored by all of the examiners.

3.3.2 Image processing prior to digital subtraction

Each of the 160 digital radiographs underwent image processing using Corel PaintShop Photo Pro X3 prior to digital subtraction. The following image processing modifications were applied to each digital radiograph in the following order:

1. The digital radiographic image was rotated to the correct anatomical orientation.
2. The digital radiographic image was converted into a 256 grey scale image.
3. The ‘blur more’ image processing tool was applied to the digital radiographic image.
4. The digital radiographic image was resized to 860x570 pixels.
5. The digital radiographic image was saved with the same identifying file name as a .bmp file.

3.3.3 Digital subtraction

Digital subtraction images were produced using Compare Software (Dental Health Unit, University of Manchester, UK) which runs as a plug-in to Image Tool (version 1.23, University of San Antonio, Texas). Fifteen digital subtraction images were produced for each molar tooth by subtracting each of the three 0 degree angulation post-demineralisation digital radiographs taken after 12, 18 and 24 hours from each of the five pre-demineralisation digital radiographs taken with 0 degree, 7 degree horizontal, 15 degree horizontal, 10 degree vertical and 15 degree vertical angulation changes in X-ray source subject projection geometry. Six-hundred digital subtraction images were produced as each molar tooth was considered separately.

Each digital subtraction image was produced using the process described below which is modified from the guidance provided with the software. To aid understanding, and for illustration purposes, Figure 11 presents the series of screenshots that were taken from the program during the production of a 0 degree angulation 24 hour digital subtraction image for the lower left second molar tooth in head three.

3.3.3.1 Digital subtraction image processing protocol

1. The appropriate pre-demineralisation and post-demineralisation digital radiograph files were opened within the Image Tool program (Figure 11 A).

2. The **Compare** command was selected and the pre-demineralisation digital radiograph file selected as the ‘1st Image’ (Figure 11 B) and the post-demineralisation digital radiograph file selected as the ‘2nd image’ (Figure 11 C).
3. **OK** was selected which opened up a new dialogue box titled ‘Compare’ (Figure 11 D).
4. Preliminary alignment of the two digital radiographic images was carried out by selecting two corresponding points on both the before and after images. This was done by selecting **‘Select set of points #1’** from the ‘Compare’ dialogue box (Figure 11 D) and selecting the mesial and distal ACJ on the molar tooth being processed on the ‘Before image’ (Figure 11 E). This was carried out for the ‘After image’ by selecting **‘Select set of points #2’** from the ‘Compare’ dialogue box (Figure 11 D) and selecting the corresponding mesial and distal ACJ on the molar tooth being processed on the ‘After image’ (Figure 11 F).
5. **‘Preliminary Warp’** was then selected from the ‘Compare’ dialogue box (Figure 11 G).
6. The **Add** button for ‘Im.1’ was selected from the ‘Compare’ dialogue box (Figure 11 H) and a polygon drawn around the crown of the molar tooth being analysed to select areas common to both digital radiographic images that were likely to have remained unchanged (Figure 11 I). The **Exclude** button for ‘Im.1’ was selected from the ‘Compare’ dialogue box (Figure 11 H) and a polygon drawn around any areas within the previously selected area that were not likely to be common to both digital radiographic images and may have changed, such as the occlusal cavity in this research project (Figure 11 J). The areas were selected on the ‘Before image’, but are displayed on both the ‘Before image’ and ‘After image’. **Warp** was then selected from the ‘Compare’ dialogue box (Figure 11 H).
7. The ‘Difference pixel jump’ value within the ‘Warp parameters’ dialogue box (Figure 11 K) was changed from the default setting of ‘20’ to ‘5’. The default settings for ‘Rotation + Translation’ and ‘Affine’ were left unchanged.
8. **Density Normalise** was selected from the ‘Compare’ dialogue box and the **Non parametric equalisation** box selected from the ‘Density

Normalisation' dialogue box (Figure 11 L). All other default settings within the 'Density Normalisation' dialogue box remained unchanged. **OK** was then selected.

9. The 'Difference image' was selected (Figure 11 M) and the **Contrast/Brightness** button selected from the **Quick access toolbar**. The **contrast slider bar** (Figure 11 N) was adjusted as required until a satisfactory 'Difference Image' was produced (Figure 11 O).
10. The 'Difference image' was selected and saved (Figure 11 P) as a .bmp image (860x570 pixels, bit depth 8) with file names to enable identification of:
    - I. Which molar tooth was used to produce the digital subtraction image;
    - II. Which X-ray source subject projection geometry was used to produce the pre-demineralisation digital radiograph; and
    - III. After which length of time the post-demineralisation digital radiograph was taken.

The procedure detailed above was repeated to produce 600 digital subtraction images for every combination of X-ray source subject projection geometry and length of demineralisation for each of the 40 molar teeth.

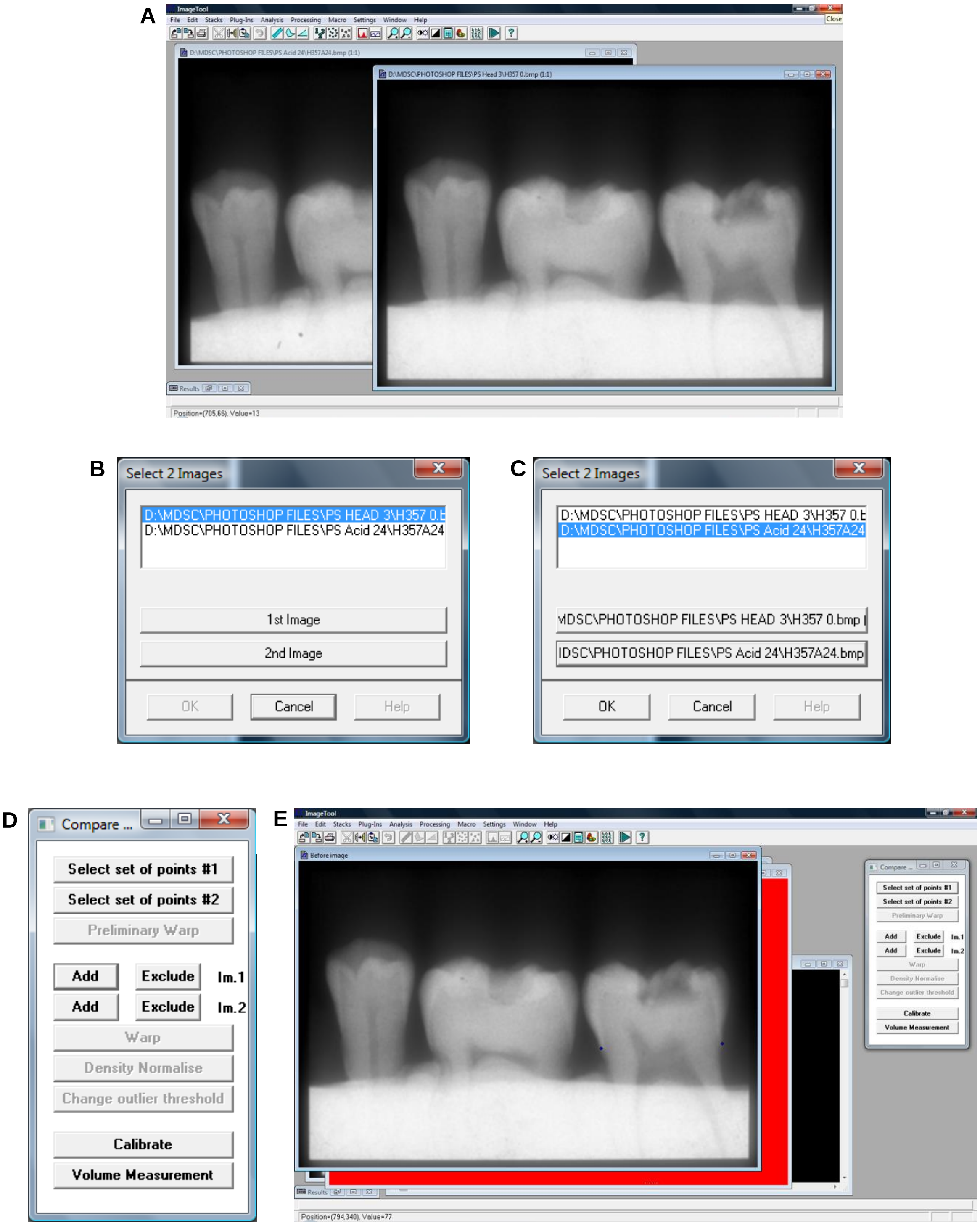


Figure 11. Screenshots taken from the Compare Software demonstrating the protocol for processing the 0 degree angulation 24 hour digital subtraction image for the lower left second molar tooth in head three. **A** Pre-demineralisation and post-demineralisation digital radiographs opened within the Image Tool program, **B** Selection of pre-demineralisation digital radiograph as ‘1st image’, **C** Selection of post-demineralisation digital radiograph as ‘2nd image’, **D** ‘Compare’ dialogue box, **E** Selection of the mesial and distal ACJ on the ‘Before image’

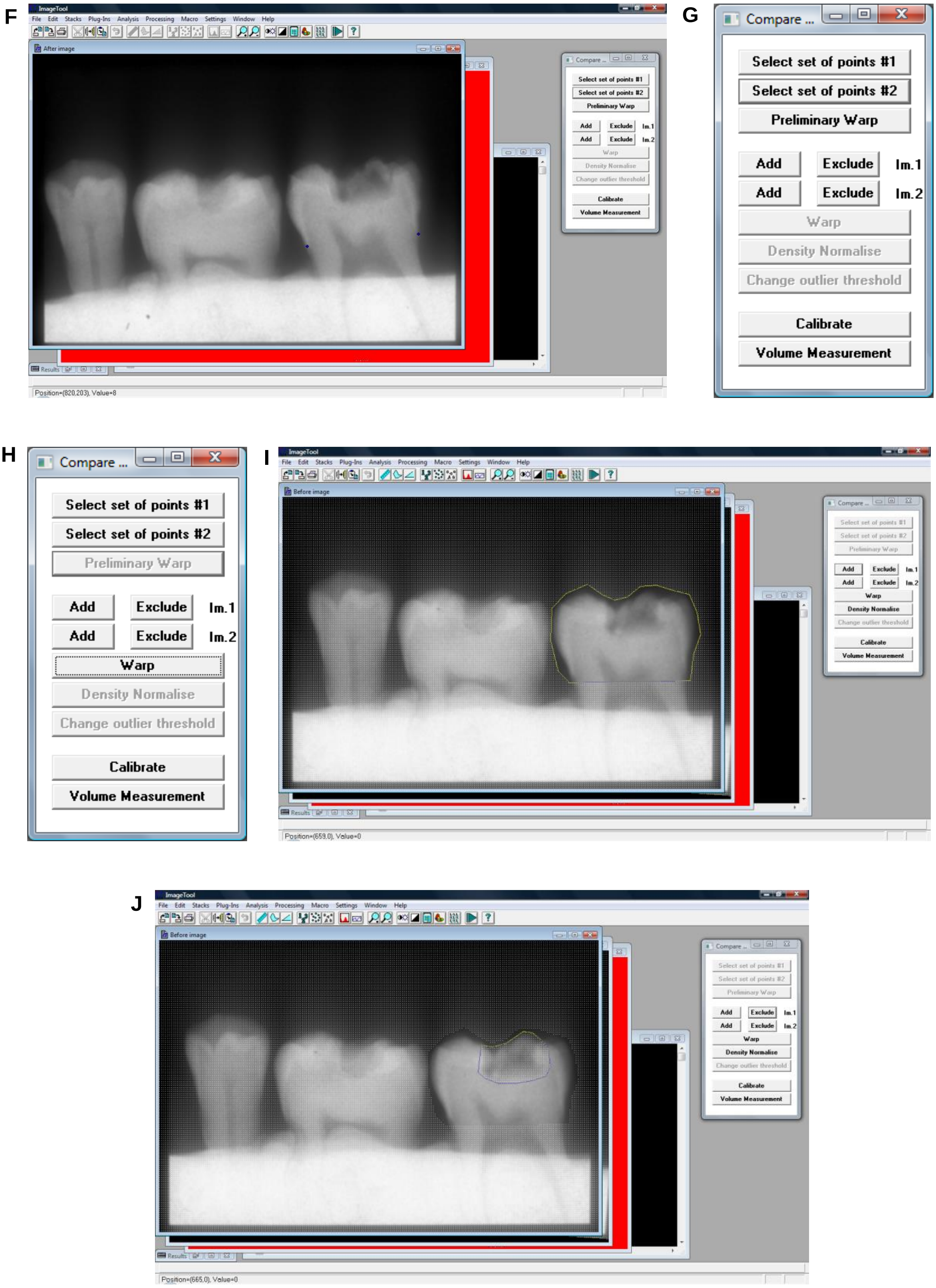


Figure 11. (continued) **F** Selection of the mesial and distal ACJ on the ‘After image’, **G** Selection of ‘Preliminary Warp’ from the ‘Compare’ dialogue box, **H** Selection of ‘Add’ and ‘Exclude’ for ‘Im.1’ from the ‘Compare’ dialogue box and selection of ‘Warp’, **I** Polygon drawn around crown of molar tooth on ‘Before image’ to select and add areas common to both digital radiographic images that are likely to have remained unchanged, **J** Polygon drawn around crown of molar tooth on ‘Before image’ to select and exclude areas that are not likely to be common to both digital radiographic images that are likely to have changed

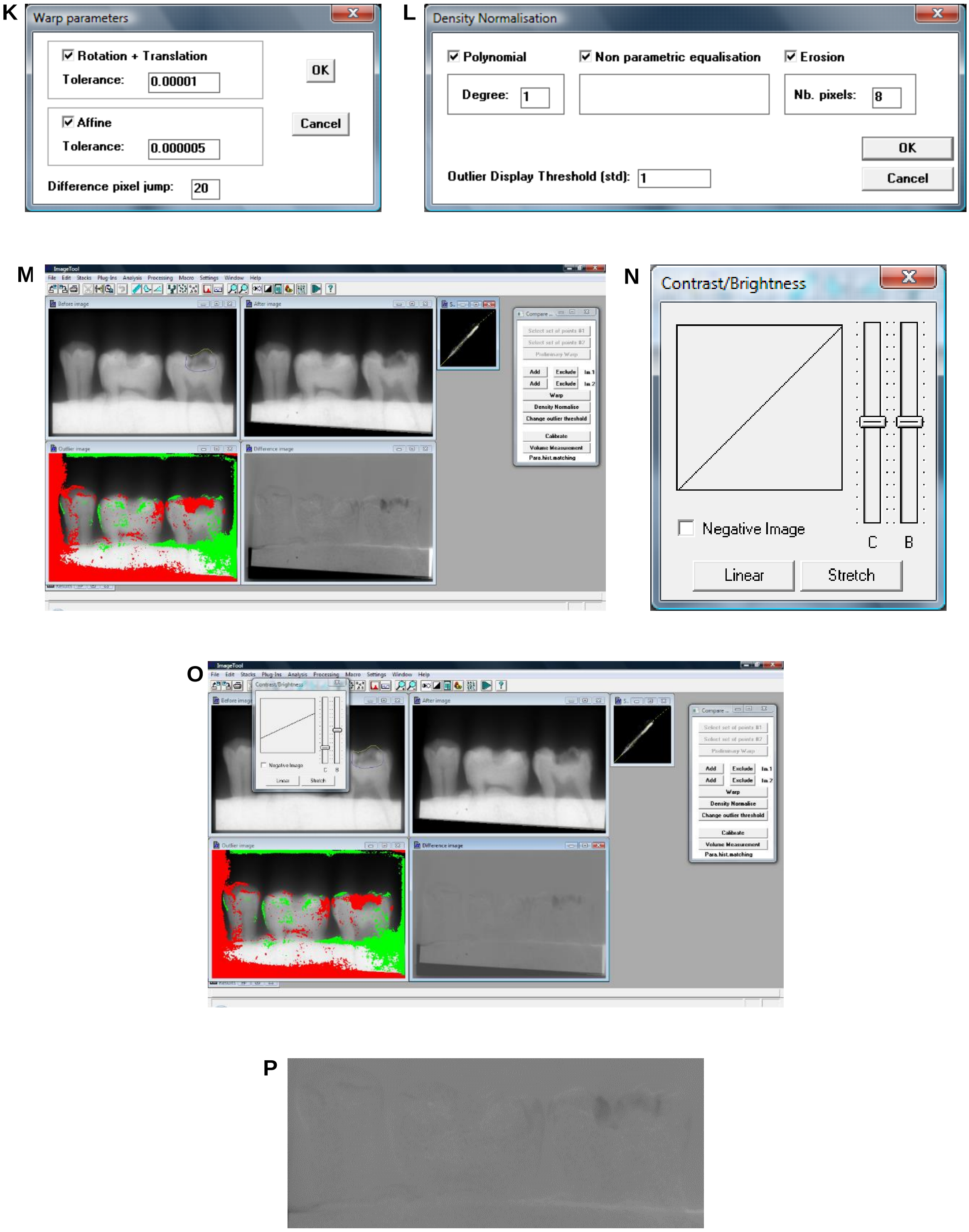


Figure 11. (continued) **K** ‘Warp parameters’ dialogue box, **L** ‘Density Normalisation’ dialogue box, **M** Selection of the ‘Difference image’, **N** Contrast slider bar, **O** Adjustment of contrast slider bar to produce satisfactory ‘Difference image’, **P** ‘Difference image’ saved as a .bmp file

3.4 Randomisation and presentation of the digital subtraction images

The 600 digital subtraction .bmp files were numbered from 1 to 600. A random sequence generator (www.random.org) was used to create a random sequence of whole numbers from 1 to 600. The digital subtraction .bmp files were inserted into a Microsoft PowerPoint (Microsoft® Office PowerPoint® 2007) presentation in the order produced by the random sequence generator. The background colour of each slide was set to black and one digital subtraction file inserted in the centre of each slide. The properties of the digital subtraction images were not altered other than cropping the image as required. The molar tooth used to produce each digital subtraction image was written at the top of each slide (Figure 12).

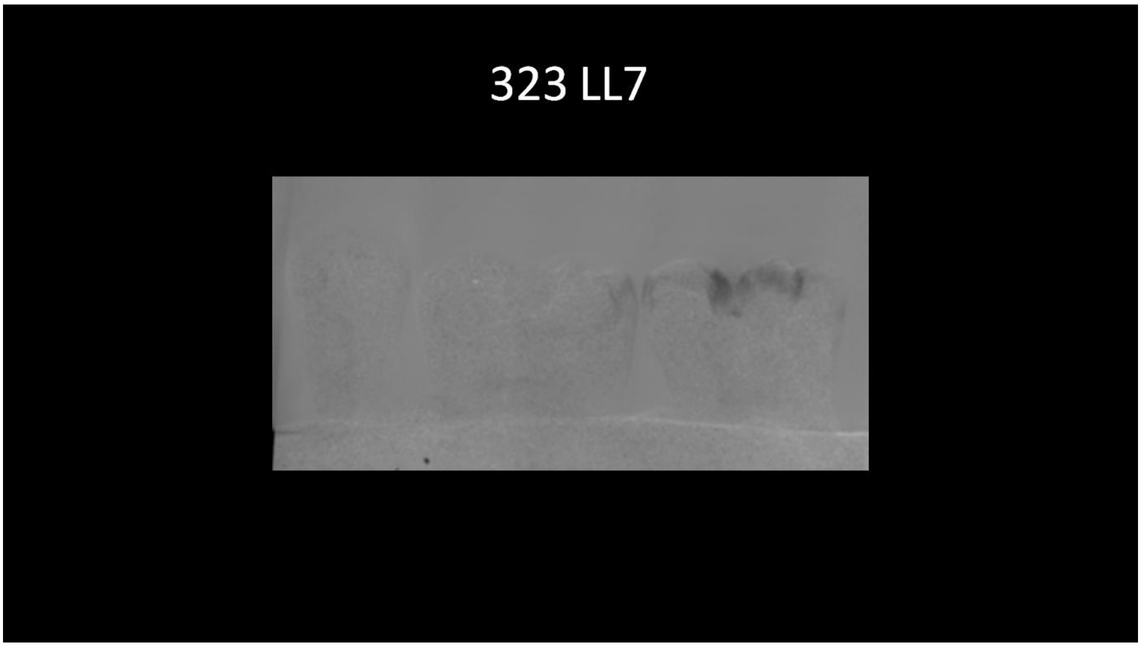


Figure 12. Microsoft PowerPoint slide number 323 for 24 hour 0 degree angulation digital subtraction image of the lower left second molar tooth in head three

3.5 Examiner inclusion criteria and calibration

3.5.1 Examiner inclusion criteria

The examiner inclusion criteria stipulated that an examiner had to:

1. be a qualified dentist;

2. be registered (as required) to practice dentistry within their country of work;
3. have experience interpreting digital radiographs as part of the clinical practice of dentistry or *in-vitro* or *in-vivo* research; and
4. be able to score the digital subtraction images within the time allocation required to enable completion of the research project.

A Participant Information Sheet was sent to all prospective examiners informing them what their participation in the research project would involve (Appendix I). Twelve examiners were recruited, to score the digital subtraction images and each examiner completed an online Participant Questionnaire (Appendix II). The examiners were chosen to represent a breadth of experience regarding the number of years they have been qualified as a dentist, their experience in interpreting digital radiographs and/or digital subtraction images, their primary area or speciality of dentistry that they work in and the proportion of their time that they spend providing clinical care to patients.

3.5.2 Calibration of examiners

An Introduction to Subtraction Radiography Information Sheet (Appendix III) was provided to each examiner. This provided information regarding the background of DSR, the materials and methods used in this research project and guidance on how to interpret the digital subtraction images produced in this research project. No formal training regarding the interpretation of digital subtraction images or calibration of examiners regarding the detection of demineralisation within the occlusal cavities in the teeth used in this research project was carried out.

3.6 Standardisation and scoring of the digital subtraction images

3.6.1 Standardisation

Examiners were instructed to sit in a comfortable position with the computer monitor at the correct height and distance from their eyes to prevent strain and that to prevent fatigue, not to look at the digital subtraction images for any longer than 30 minutes in each individual session. There was no standardisation for type of computer monitor used, screen settings, or surrounding ambient environment as the examiners lived in a variety of countries worldwide.

3.6.2 Scoring the digital subtraction images

Each examiner independently scored each digital subtraction image as to their level of certainty that occlusal demineralisation of the indicated molar tooth had taken place. A five-point certitude scale was used:

1. Definitely no demineralisation
2. Likely no demineralisation
3. Do not know
4. Likely demineralisation
5. Definite demineralisation

The scores were recorded on a Microsoft Word document (Microsoft® Word 2007) ‘Data collection sheet – 600 images’ (Appendix IV) along with the examiners name and date of completion and e-mailed to the principle researcher, SR.

3.7 Assessment of reproducibility

One-hundred digital subtraction files were selected from the original 600 .bmp digital subtraction files using a random sequence generator (www.random.org) to enable assessment of intra-examiner reproducibility. The 100 digital subtraction .bmp files were inserted into a new Microsoft PowerPoint (Microsoft® Office PowerPoint® 2007) presentation in an order produced by a random sequence generator (www.random.org) in exactly the same format as the original whole sample and viewed in exactly the same manner.

Each examiner scored the 100 digital subtraction images independently one month after they had completed scoring the previous 600 digital subtraction images using the same five-point certitude scale. The scores were recorded on a Microsoft Word document (Microsoft® Word 2007) ‘Data collection sheet – Reproducibility’ (Appendix V) along with the examiners name and date of completion and e-mailed to the principle researcher, SR.

3.8 Statistical analysis

3.8.1 Accuracy of detection of demineralisation in occlusal cavities

ROC analysis was carried out for each examiner for each angulation and length of demineralisation using IBM SPSS Statistics Version 20. Univariate analysis of variance of the mean AuROC curve for all 12 examiners at each angulation and length of demineralisation was carried out, in addition to one-way analysis of variance of the mean AuROC curve for all 12 examiners at each angulation after 12, 18 and 24 hours demineralisation. Post-hoc tests for multiple comparisons of the effect that each variation in angulation had on the mean AuROC curve for all 12 examiners after 12, 18 and 24 hours demineralisation using a Bonferroni correction was carried out. Parametric paired samples t-test was used to test for statistically significant differences between the mean AuROC curve for all 12 examiners comparing a distal shift with a mesial shift in X-ray source following a 7 degree and 15 degree horizontal angulation variation in X-ray source subject projection geometry after 12, 18 and 24 hours demineralisation. Parametric paired samples t-test was used to test for statistically significant differences between the mean AuROC curve for all 12 examiners combined comparing a positive upward shift with a negative downward shift in X-ray source following a 10 degree and 15 degree vertical angulation variation in X-ray source subject projection geometry after 12, 18 and 24 hours of demineralisation.

3.8.2 Reproducibility of detection of demineralisation in occlusal cavities

Intra- and inter-examiner reproducibility was calculated using weighted kappa and percentage agreement.

Part 2: The discriminatory ability and reproducibility of a grading system for assessing observed changes in the proximal relationships of teeth on paired digital radiographs taken with horizontal variations in the X-ray source subject projection geometry

3.9 Introduction

The grading system was designed to allow recording of the size of the inter-proximal space or degree of overlap of contact points between teeth on bitewing radiographs that would be useable in a clinical setting as well as being used in the study. Sixty of the initial 160 digital radiographs were used for Part 2 of this research project. The 60 digital radiographs comprised three sets of radiographs for the 20 posterior dental arch sextants discussed in Part 1 as follows:

- the 20, 0 degree angulation 12 hour post-demineralisation digital radiographs,
- the 20, 7 degree horizontal angulation pre-demineralisation digital radiographs; and
- the 20, 15 degree horizontal angulation pre-demineralisation digital radiographs.

Section 3.2 described the materials and methods used to produce these 60 digital radiographs and section 3.3.2 described the image processing that was carried out to produce the .bmp files prior to digital subtraction.

A flowchart summarising the materials and methods for Part 2 of this research project is illustrated in Figure 13.

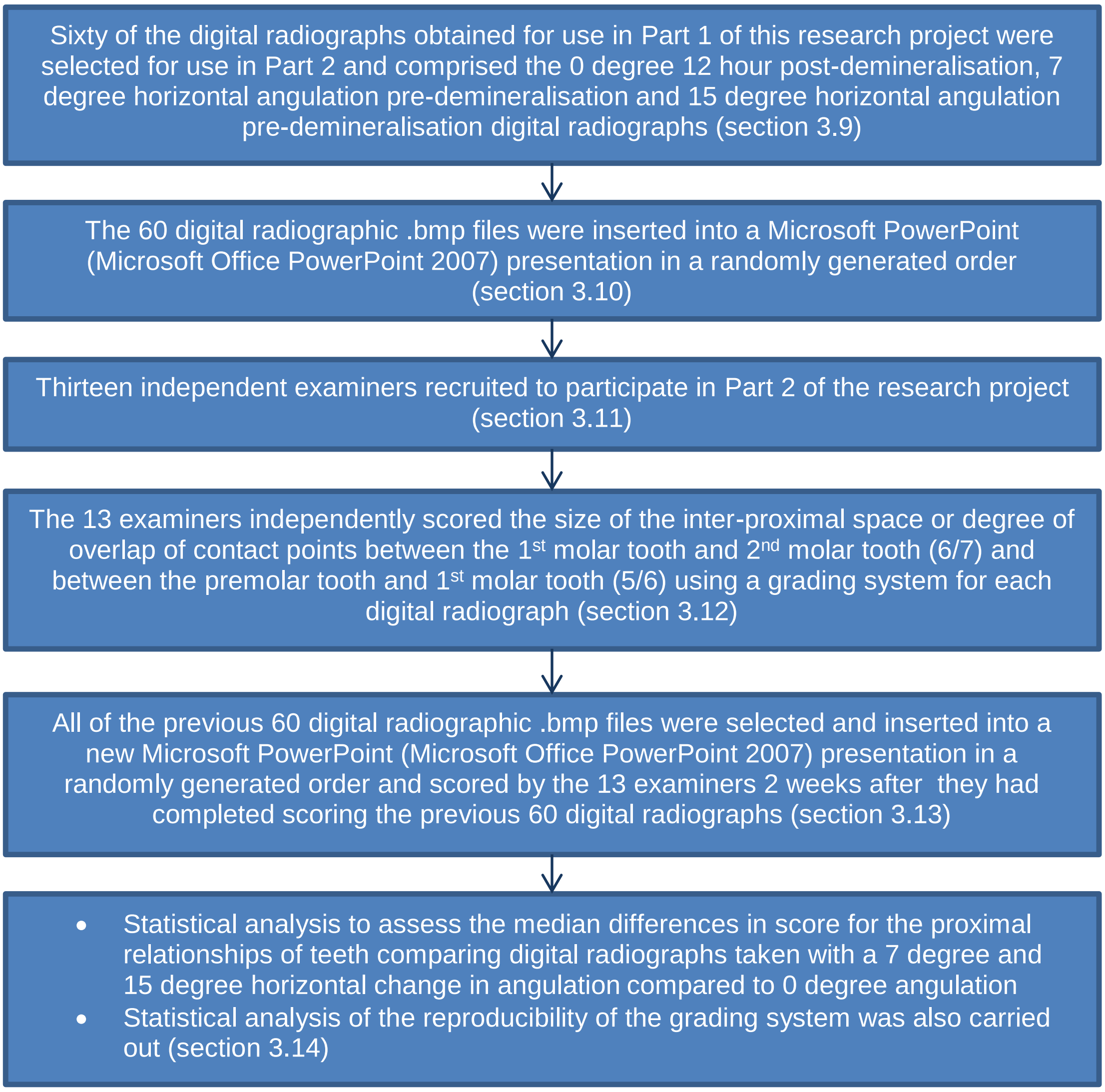


Figure 13. Flowchart summarising the materials and methods for Part 2 of this research project

3.10 Randomisation and presentation of the digital radiographs

The 60 digital radiographic .bmp files were numbered from 1 to 60. A random sequence generator (www.random.org) was used to create a random sequence of whole numbers from 1 to 60. The 60 digital radiographic .bmp files were inserted into a Microsoft PowerPoint (Microsoft® Office PowerPoint® 2007) presentation in the order produced by the random sequence generator. The background colour of each slide was set to black and one digital radiographic .bmp file inserted in the centre of each slide. The properties of the digital radiographs were not altered. The slides were numbered from 1 to 60 and this was written at the top of each slide (Figure 14).

Figure 14. Microsoft PowerPoint slide number 34 presenting the 12 hour 0 degree post-demineralisation digital radiograph for the lower left posterior dental arch sextant in head three

3.11 Examiner inclusion criteria and calibration

3.11.1 Examiner inclusion criteria

The examiners were purposively sampled to represent a range of:

- years qualified as a dentist;
- experience in interpreting digital radiographs;
- primary area or speciality of dentistry; and
- the proportion of time spent providing clinical care to patients.

The examiner inclusion criteria were identical to that in Part 1 of this study (see section 3.5.1), with the exception of an amendment to number 4 which read 'be able to score the digital radiographs within the time allocation required to enable completion of the research project'. A Participant Information Sheet was sent to all prospective examiners informing them of what participation would involve (Appendix VI). Out of 13 examiners invited to participate, all 13 agreed. These 13 examiners scored the digital radiographs and completed an online Participant Questionnaire (Appendix II) if they had not participated in Part 1 of the project.

3.11.2 Calibration of examiners

The Participant Information Sheet (Appendix VI) informed examiners that they would have to score the size of inter-proximal space or degree of overlap of contact points between teeth on digital radiographs using a grading system. An explanation of the grading system criteria was provided to each examiner on the front page of the data collection sheets, Microsoft Word document (Microsoft® Word 2007) ‘Overlap assessment – Initial 60’ (Appendix VII) and Microsoft Word document (Microsoft® Word 2007) ‘Overlap assessment - Reproducibility’ (Appendix VIII) sent to each examiner.

To allow assessment of the usability of the grading system without training or calibration (as is likely to happen in the clinical setting), there was no formal training or calibration of examiners in its use.

3.12 Standardisation and scoring of the digital radiographs

3.12.1 Standardisation

Examiners were instructed to sit in a comfortable position with the computer monitor at the correct height and distance from their eyes to prevent strain and fatigue. There was no standardisation for type of computer monitor used, screen settings, or surrounding ambient environment as the examiners lived in a variety of countries worldwide.

3.12.2 Scoring the digital radiographs

Each examiner independently scored the size of the inter-proximal space or degree of overlap of contact points between the 6/7 and between the 5/6 using the grading system described in Figure 15.

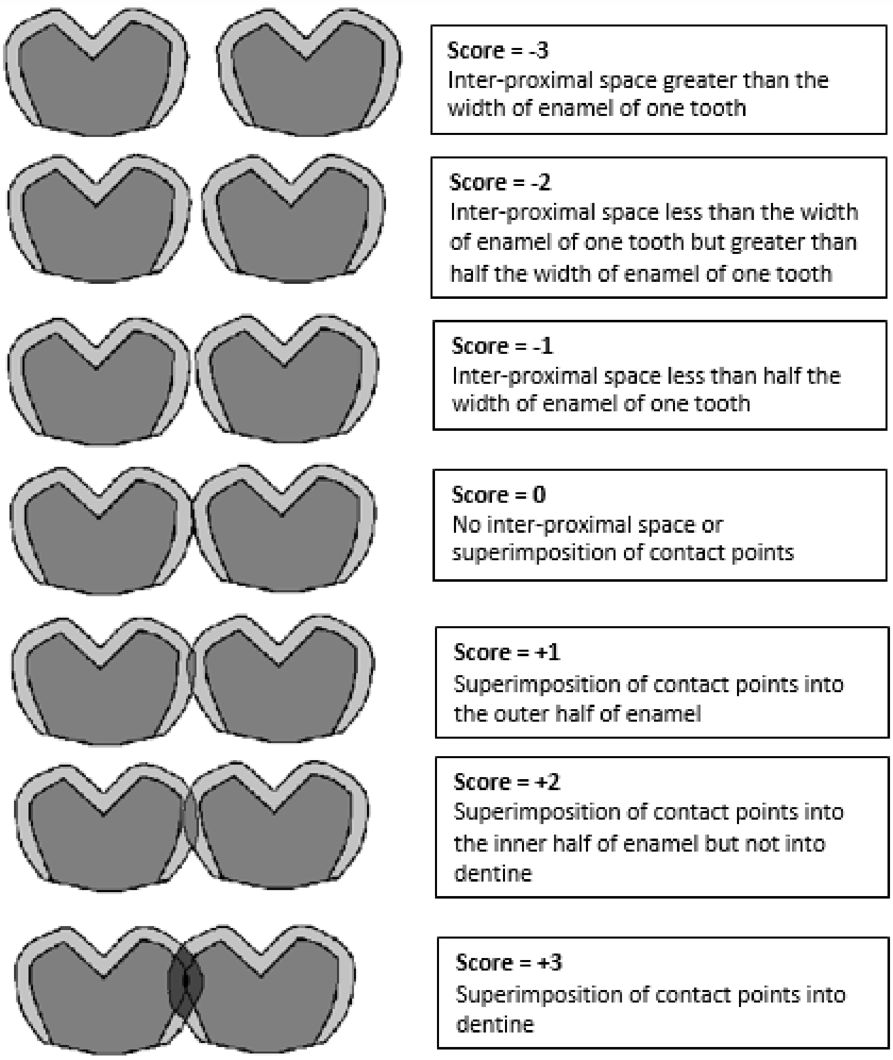


Figure 15. Grading system to assess the proximal relationship of teeth to one another

The scores were recorded on a Microsoft Word document (Microsoft® Word 2007) 'Overlap assessment – Initial 60' (Appendix VII) along with the examiners name and date of completion and e-mailed to the principal researcher, SR.

3.13 Assessment of reproducibility

The 60 digital radiographs were re-examined to enable assessment of intra-examiner reproducibility. A random sequence generator (www.random.org) was used to create a new random sequence of whole numbers from 1 to 60 and the digital radiographic .bmp files were handled, viewed and scored in exactly the same manner as for the first examination. The scores were recorded on a

Microsoft Word document (Microsoft® Word 2007) ‘Overlap assessment – Reproducibility’ (Appendix VIII) along with the examiners name and date of completion and e-mailed to the principal researcher, SR.

3.14 Statistical analysis

The median differences in scores recorded for the proximal relationships between the 6/7, and between the 5/6 were calculated for each examiner, and for all 13 examiners comparing digital radiographs taken with a 7 degree and 15 degree horizontal angulation variation compared to 0 degree angulation. Intra- and inter-examiner reproducibility was calculated using weighted kappa and percentage agreement.

4 Results

Part 1: *In-vitro* the accuracy and reproducibility of DSR for detecting demineralisation in occlusal cavities using digital radiographs taken with variations in X-ray source subject projection geometry after varying time periods of demineralisation

4.1 Diagnostic quality of the digital subtraction images

Out of the 600 digital subtraction images produced, 12 images were not of diagnostic quality. These were due to a random positioning error associated with the PSP plate for the 15 degree horizontal angulation pre-demineralisation digital radiograph for one of the posterior dental arch sextants and a random digital processing error associated with the 0 degree angulation pre-demineralisation digital radiograph for another posterior dental arch sextant resulting in the production of a double image.

This resulted in 588 digital subtraction images for use in Part 1 of this research project.

4.2 Demographics of the 12 examiners

The 12 examiners had a mean age of 41.5 years (range 27 to 56) with a mean of 18.5 years (range 5 to 31) as a qualified dentist. Only one was a specialist in Dental and Maxillofacial Radiology, however all were confident in viewing and interpreting intra-oral radiographs for detecting and monitoring carious lesions, and half had also had previous experience of and felt confident in the interpretation of subtraction radiography. The demographics of the 12 independent examiners are displayed in Table 6.

Table 6. Demographics of the 12 examiners who participated in Part 1

Age (years)	
Median	42.5
Mean	41.5
Range	27 - 56
Sex	
Male : Female	6(50%) : 6(50%)
Years qualified as a dentist	
Median	17.5
Mean	18.5
Range	5 - 31
Registered specialist in Dental and Maxillofacial Radiology	
Yes : No	1(8%) : 11(92%)
Number of intra-oral radiographs viewed per week (clinical and research)	
Median	20
Mean	21.7
Range	0 - 50
Percentage of intra-oral radiographs viewed that involve the detection and/or monitoring of carious lesions (clinical and research)	
Median	90%
Mean	68%
Range	0% - 100%
Confidence interpreting intra-oral radiographs (0 = not at all confident, 10 = very confident)	
Median	8
Mean	8.1
Range	7 - 10
Confidence using intra-oral radiographs for detecting and/or monitoring carious lesions (0 = not at all confident, 10 = very confident)	
Median	8
Mean	7.5
Range	5 - 9
Previous experience using DSR (clinical and research)	
Yes : No	6(50%) : 6(50%)
Previous experience using DSR for detecting and/or monitoring carious lesions (clinical and research)	
Yes : No	6(50%) : 6(50%)
Confidence interpreting digital subtraction images (0 = not at all confident, 10 = very confident)	
Median	6.5
Mean	6.4
Range	4 - 9
Confidence using DSR for detecting and/or monitoring carious lesions (0 = not at all confident, 10 = very confident)	
Median	6.5
Mean	6.5
Range	4 - 9

4.3 Assessment of accuracy for detecting demineralisation in occlusal cavities

Each independent examiner graded their level of certainty as to the presence of demineralisation in the occlusal cavity of each molar tooth using the five-point certitude scale. The scores were analysed and validated against the true presence of demineralisation, which was the placement of demineralising solution into the occlusal cavities. This data was used to produce ROC curves using IBM SPSS Statistics Version 20 for each examiner at each angulation and length of demineralisation, and the AuROC curves for each of the 12 examiners are shown in Table 7.

The mean AuROC curve was calculated for all 12 examiners at each angulation and length of demineralisation and is shown in Table 8 along with the standard deviation, standard error, 95% confidence intervals and, minimum and maximum values.

Table 7. AuROC curve for each examiner at each angulation and length of demineralisation

Angulation	Length of demineralisation	AuROC curve											
		Ex 1	Ex 2	Ex 3	Ex 4	Ex 5	Ex 6	Ex 7	Ex 8	Ex 9	Ex 10	Ex 11	Ex 12
0 degrees	12 hours	0.940	0.828	0.821	0.889	0.882	0.805	0.856	0.812	0.849	0.889	0.873	0.857
7 degrees horizontal	12 hours	0.900	0.835	0.865	0.841	0.793	0.845	0.816	0.801	0.910	0.662	0.852	0.792
15 degrees horizontal	12 hours	0.750	0.614	0.639	0.658	0.697	0.574	0.642	0.583	0.751	0.578	0.601	0.614
15 degrees vertical	12 hours	0.768	0.684	0.697	0.752	0.664	0.719	0.634	0.640	0.609	0.719	0.624	0.650
10 degrees vertical	12 hours	0.764	0.697	0.649	0.777	0.663	0.684	0.729	0.665	0.721	0.613	0.583	0.708
0 degrees	18 hours	1.000	0.888	0.904	0.881	0.931	0.914	0.946	0.988	0.953	0.970	0.875	0.839
7 degrees horizontal	18 hours	0.881	0.709	0.777	0.793	0.771	0.846	0.741	0.796	0.786	0.806	0.762	0.768
15 degrees horizontal	18 hours	0.800	0.649	0.665	0.758	0.686	0.681	0.647	0.779	0.760	0.660	0.642	0.661
15 degrees vertical	18 hours	0.752	0.565	0.704	0.719	0.610	0.575	0.672	0.613	0.749	0.580	0.689	0.669
10 degrees vertical	18 hours	0.806	0.738	0.663	0.781	0.727	0.668	0.816	0.789	0.739	0.761	0.679	0.828
0 degrees	24 hours	0.972	0.947	0.921	0.947	0.934	0.968	0.877	1.000	0.877	0.997	0.931	0.981
7 degrees horizontal	24 hours	0.996	0.845	0.843	0.893	0.934	0.916	0.807	0.950	0.965	0.826	0.921	0.950
15 degrees horizontal	24 hours	0.794	0.594	0.606	0.918	0.804	0.785	0.775	0.775	0.876	0.610	0.714	0.721
15 degrees vertical	24 hours	0.766	0.658	0.699	0.846	0.723	0.614	0.712	0.718	0.654	0.678	0.689	0.771
10 degrees vertical	24 hours	0.873	0.707	0.746	0.863	0.746	0.842	0.843	0.784	0.831	0.851	0.762	0.847

Table 8. Mean AuROC curve and descriptive statistics for the 12 examiners at each angulation and length of demineralisation

Angulation	Length of demineralisation	Mean AuROC curve	Standard deviation	Standard error	95% Confidence interval for mean		Minimum	Maximum
					Lower bound	Upper bound		
0 degrees	12 hours	0.858	0.039	0.011	0.834	0.883	0.805	0.940
7 degrees horizontal	12 hours	0.826	0.064	0.019	0.785	0.867	0.662	0.910
15 degrees horizontal	12 hours	0.642	0.062	0.018	0.602	0.681	0.574	0.751
15 degrees vertical	12 hours	0.680	0.052	0.015	0.647	0.713	0.609	0.768
10 degrees vertical	12 hours	0.688	0.057	0.017	0.651	0.724	0.583	0.777
0 degrees	18 hours	0.924	0.049	0.014	0.893	0.955	0.839	1.000
7 degrees horizontal	18 hours	0.786	0.045	0.013	0.758	0.815	0.709	0.881
15 degrees horizontal	18 hours	0.699	0.058	0.017	0.662	0.736	0.642	0.800
15 degrees vertical	18 hours	0.658	0.068	0.019	0.615	0.701	0.565	0.752
10 degrees vertical	18 hours	0.750	0.057	0.017	0.713	0.786	0.663	0.828
0 degrees	24 hours	0.946	0.041	0.012	0.920	0.972	0.877	1.000
7 degrees horizontal	24 hours	0.904	0.061	0.018	0.865	0.942	0.807	0.996
15 degrees horizontal	24 hours	0.748	0.104	0.030	0.682	0.814	0.594	0.918
15 degrees vertical	24 hours	0.711	0.062	0.018	0.671	0.750	0.614	0.846
10 degrees vertical	24 hours	0.808	0.056	0.016	0.773	0.843	0.707	0.873

4.3.3 Effect of variations in X-ray source subject projection geometry on the accuracy for detecting demineralisation in occlusal cavities

Univariate analysis of variance of the mean AuROC curve for all 12 examiners identified that there was a statistically significant interaction between variations in angulation and length of demineralisation (F=2.778, P<0.01).

One-way analysis of variance of the mean AuROC curve for all 12 examiners found that variations in angulation resulted in statistically significant differences after 12 hours (F=36.319, P<0.001), 18 hours (F=40.000, P<0.001) and 24 hours (F=26.156, P<0.001) demineralisation.

Post-hoc tests for multiple comparisons of the effect each variation in angulation had on the mean AuROC curve for all 12 examiners after 12 hours, 18 hours and 24 hours demineralisation using a Bonferroni correction was carried out.

After 12 hours and 24 hours demineralisation, comparing 7 degrees horizontal angulation to 0 degrees angulation, there were no statistically significant differences (P=1.000) in the mean AuROC curve for all 12 examiners. However, there were statistically significant differences (P<0.001) when comparing 15 degrees horizontal, 15 degrees vertical and 10 degrees vertical angulation to 0 degrees angulation.

After 18 hours, there were statistically significant differences (P<0.001) comparing 7 degrees horizontal, 15 degrees horizontal, 15 degrees vertical and 10 degrees vertical angulation to 0 degrees angulation.

4.3.4 Effect of mesial compared to distal variation in horizontal X-ray source subject projection geometry on the accuracy for detecting demineralisation in occlusal cavities

The mean AuROC curve for each examiner and all 12 examiners was calculated for the upper right and lower left posterior dental arch sextants (which represented a distal shift in the horizontal angulation of the X-ray source) and the upper left and lower right posterior dental arch sextants (which represented a mesial shift in the horizontal angulation of the X-ray source) for a 7 degree horizontal and 15 degree horizontal angulation variation in X-ray source subject projection geometry compared to 0 degree angulation after 12, 18 and 24 hours demineralisation (Table 9).

Paired samples t-test was used to test for statistically significant differences between the mean AuROC curve for all 12 examiners comparing a distal shift in the horizontal angulation of the X-ray source to a mesial shift in the horizontal angulation of the X-ray source for both a 7 degree and 15 degree angulation variation in X-ray source subject projection geometry compared to 0 degree angulation after 12, 18 and 24 hours of demineralisation. No statistically significant differences ($P \geq 0.05$) were identified comparing a distal shift to a mesial shift in the horizontal angulation of the X-ray source for a 7 degree variation compared to 0 degree angulation after 12, 18 and 24 hours demineralisation and for a 15 degree variation compared to 0 degree angulation after 18 hours demineralisation. A statistically significant difference ($P < 0.01$) was however identified favouring a distal shift in horizontal angulation of the X-ray source for a 15 degree angulation variation compared to 0 degree angulation after 12 and 24 hours demineralisation.

Table 9. Mean AuROC curve following a mesial and distal shift in the horizontal angulation of the X-ray source for a 7 degree horizontal and 15 degree horizontal angulation variation in X-ray source subject projection geometry compared to 0 degree angulation after 12, 18 and 24 hours demineralisation (n=12 examiners)

Sextant	Angulation	Length	Mean AuROC curve												
			Ex1	Ex2	Ex3	Ex4	Ex5	Ex6	Ex7	Ex8	Ex9	Ex10	Ex11	Ex12	All 12 examiners
UR + LL	7 degrees horizontal - distal	12 hours	1.000	0.828	0.874	0.874	0.904	0.081	0.904	0.904	0.894	0.657	0.854	0.798	0.798
UL + LR	7 degrees horizontal - mesial	12 hours	0.770	0.845	0.855	0.810	0.064	0.895	0.740	0.695	0.920	0.650	0.850	0.785	0.740
UR + LL	7 degrees horizontal - distal	18 hours	0.990	0.682	0.773	0.939	0.803	0.833	0.798	0.848	0.712	0.869	0.717	0.803	0.814
UL + LR	7 degrees horizontal - mesial	18 hours	0.790	0.730	0.770	0.670	0.735	0.860	0.685	0.760	0.885	0.780	0.815	0.735	0.768
UR + LL	7 degrees horizontal - distal	24 hours	1.000	0.889	0.884	0.934	0.944	0.955	0.904	0.934	0.970	0.803	0.970	0.985	0.931
UL + LR	7 degrees horizontal - mesial	24 hours	0.990	0.800	0.800	0.850	0.925	0.885	0.695	0.975	0.970	0.910	0.885	0.910	0.883
UR + LL	15 degrees horizontal - distal	12 hours	0.825	0.700	0.700	0.750	0.763	0.563	0.725	0.569	0.719	0.638	0.600	0.788	0.695
UL + LR	15 degrees horizontal - mesial	12 hours	0.660	0.530	0.580	0.560	0.620	0.570	0.555	0.600	0.775	0.515	0.575	0.430	0.581
UR + LL	15 degrees horizontal - distal	18 hours	0.750	0.650	0.650	0.994	0.794	0.663	0.737	0.838	0.719	0.656	0.813	0.706	0.748
UL + LR	15 degrees horizontal - mesial	18 hours	0.850	0.645	0.670	0.510	0.535	0.700	0.560	0.725	0.800	0.660	0.470	0.590	0.643
UR + LL	15 degrees horizontal - distal	24 hours	0.806	0.576	0.688	1.000	0.919	0.881	0.888	0.869	0.906	0.575	0.687	0.812	0.801
UL + LR	15 degrees horizontal - mesial	24 hours	0.720	0.610	0.540	0.820	0.700	0.700	0.685	0.665	0.850	0.675	0.740	0.620	0.694

4.3.5 Effect of positive upward compared to negative downward variation in vertical X-ray source subject projection geometry on the accuracy for detecting demineralisation in occlusal cavities

The mean AuROC curve for each examiner and all 12 examiners was calculated for the upper right and upper left posterior dental arch sextants when the 15 degree vertical angulation pre-demineralisation digital radiographs were used (which represented a negative downwards 15 degree vertical shift in X-ray source subject projection geometry) and the lower right and lower left posterior dental arch sextants when the 15 degree vertical angulation pre-demineralisation digital radiographs were used (which represented a positive upwards 15 degree vertical shift in X-ray source subject projection geometry) (Table 10).

The mean AuROC curve for each examiner and all 12 examiners was also calculated for the upper right and upper left posterior dental arch sextants when the 10 degree vertical angulation pre-demineralisation digital radiographs were used (which represented a positive upwards 10 degree vertical shift in X-ray source subject variation) and the lower right and lower left posterior dental arch sextants when the 10 degree vertical angulation pre-demineralisation digital radiographs were used (which represented a negative downwards 10 degree vertical shift in X-ray source subject variation) (Table 10).

Table 10. Mean AuROC curve following a positive upwards shift and negative downwards shift in the vertical angulation of the X-ray source for a 10 degree vertical and 15 degree vertical angulation variation in X-ray source subject projection geometry compared to 0 degree angulation after 12, 18 and 24 hours demineralisation (n=12 examiners)

Sextant	Angulation	Length	Mean AuROC curve												
			Ex1	Ex2	Ex3	Ex4	Ex5	Ex6	Ex7	Ex8	Ex9	Ex10	Ex11	Ex12	All 12 examiners
UL + UR	-15 degrees vertical	12 hours	0.727	0.677	0.662	0.667	0.591	0.717	0.687	0.732	0.419	0.747	0.692	0.682	0.667
LL + LR	+15 degrees vertical	12 hours	0.810	0.670	0.690	0.840	0.745	0.710	0.620	0.545	0.790	0.710	0.555	0.620	0.692
UL + UR	-15 degrees vertical	18 hours	0.652	0.379	0.606	0.783	0.485	0.581	0.490	0.753	0.753	0.540	0.712	0.732	0.622
LL + LR	+15 degrees vertical	18 hours	0.840	0.750	0.800	0.665	0.695	0.570	0.810	0.495	0.745	0.605	0.670	0.610	0.688
UL + UR	-15 degrees vertical	24 hours	0.732	0.525	0.631	0.808	0.672	0.652	0.601	0.727	0.682	0.662	0.641	0.768	0.675
LL + LR	+15 degrees vertical	24 hours	0.795	0.800	0.750	0.870	0.770	0.590	0.845	0.725	0.630	0.700	0.730	0.740	0.745
UL + UR	+10 degrees vertical	12 hours	0.692	0.828	0.747	0.768	0.631	0.687	0.697	0.717	0.702	0.571	0.702	0.747	0.707
LL + LR	-10 degrees vertical	12 hours	0.815	0.580	0.550	0.785	0.670	0.660	0.760	0.625	0.735	0.645	0.470	0.660	0.663
UL + UR	+10 degrees vertical	18 hours	0.793	0.707	0.854	0.768	0.707	0.722	0.833	0.717	0.712	0.823	0.646	0.793	0.756
LL + LR	-10 degrees vertical	18 hours	0.820	0.760	0.460	0.785	0.735	0.660	0.800	0.880	0.745	0.710	0.705	0.850	0.743
UL + UR	+10 degrees vertical	24 hours	0.838	0.763	0.864	0.889	0.808	0.793	0.838	0.793	0.818	0.793	0.753	0.843	0.816
LL + LR	-10 degrees vertical	24 hours	0.885	0.650	0.640	0.830	0.685	0.890	0.840	0.765	0.850	0.905	0.740	0.840	0.793

Paired samples t-test was used to test for statistically significant differences between the mean AuROC curve for all 12 examiners comparing a positive upward shift in the vertical angulation of the X-ray source to a negative downward shift in the vertical angulation of the X-ray source for both a 10 degree and 15 degree angulation variation in X-ray source subject projection geometry compared to 0 degree angulation after 12, 18 and 24 hours of demineralisation. No statistically significant differences ($P \geq 0.05$) were identified comparing a negative downward shift in the vertical angulation of the X-ray source compared to a positive upward shift in the vertical angulation of the X-ray source for a 10 degree variation compared to 0 degree angulation after 12, 18 and 24 hours demineralisation and for a 15 degree variation compared to 0 degree angulation after 12 and 18 hours demineralisation. A statistically significant difference ($P < 0.05$) was however identified favouring a positive upward shift compared to a negative downwards shift in vertical angulation of the X-ray source for a 15 degree angulation variation compared to 0 degree baseline after 24 hours demineralisation.

4.4 Assessment of intra-examiner reproducibility for the detection of demineralisation in occlusal cavities

One-hundred digital subtraction images were selected at random from the original 588 digital subtraction images. These were re-scored by the examiners one month later using the same five-point certitude scale as to their level of certainly that demineralisation was present in the occlusal cavity of each molar tooth. Weighted kappa values and percentage agreement were calculated to assess the intra-examiner reproducibility for each of the 12 examiners for detecting occlusal demineralisation (Table 11).

Table 11. Weighted kappa values and percentage agreement for intra-examiner reproducibility (n=12 examiners). The weighted kappa values have been colour coded according to their suggested interpretation as described by Landis and Koch (1977); <0 = Poor agreement, 0.01 - 0.20 = Slight agreement, 0.21 – 0.40 = Fair agreement, 0.41 – 0.60 = Moderate agreement, 0.61 – 0.80 = Substantial agreement, 0.81 – 1.00 = Almost perfect agreement

Examiner	1	2	3	4	5	6	7	8	9	10	11	12
Weighted Kappa	0.70	0.46	0.49	0.69	0.58	0.68	0.35	0.59	0.65	0.88	0.51	0.51
Percentage Agreement	91%	84%	85%	86%	84%	89%	76%	84%	89%	96%	87%	85%

For all 12 examiners, the median intra-examiner weighted kappa value was 0.585, range 0.35-0.88, interquartile range (IQR) 0.17, first quartile (Q_1) 0.51 and third quartile (Q_3) 0.68. For all 12 examiners, the median percentage agreement was 86%, range 76%-96%, IQR 5%, Q_1 84% and Q_3 89%.

4.5 Assessment of inter-examiner reproducibility for the detection of demineralisation in occlusal cavities

The weighted kappa values and percentage agreement for all angulations are presented in Table 12, for 7 degree horizontal and 0 degree angulation in Table 13 and for 0 degree angulation in Table 14.

Table 12. Weighted kappa values (upper value) and percentage agreement (lower value) for inter-examiner reproducibility at all angulations (n=12 examiners). The weighted kappa values have been colour coded according to their suggested interpretation as described by Landis and Koch (1977); <0 = Poor agreement, 0.01 - 0.20 = Slight agreement, 0.21 – 0.40 = Fair agreement, 0.41 – 0.60 = Moderate agreement, 0.61 – 0.80 = Substantial agreement, 0.81 – 1.00 = Almost perfect agreement

Examiner	2	3	4	5	6	7	8	9	10	11	12
1	0.18 62%	0.18 62%	0.50 79%	0.49 81%	0.57 85%	0.39 76%	0.53 82%	0.55 85%	0.50 82%	0.46 83%	0.51 83%
2		0.81 95%	0.24 63%	0.21 65%	0.14 57%	0.29 73%	0.23 67%	0.15 59%	0.17 65%	0.08 59%	0.22 69%
3			0.25 63%	0.21 65%	0.14 57%	0.28 73%	0.24 68%	0.15 60%	0.19 65%	0.08 60%	0.23 70%
4				0.49 78%	0.46 77%	0.38 72%	0.43 75%	0.41 75%	0.42 75%	0.30 71%	0.40 74%
5					0.48 80%	0.40 76%	0.47 79%	0.43 79%	0.45 79%	0.35 77%	0.45 80%
6						0.34 73%	0.49 81%	0.49 83%	0.48 81%	0.37 80%	0.38 78%
7							0.41 77%	0.32 73%	0.35 75%	0.20 71%	0.38 77%
8								0.48 80%	0.53 82%	0.36 78%	0.48 81%
9									0.46 80%	0.34 79%	0.40 79%
10										0.37 79%	0.48 82%
11											0.37 80%

The median inter-examiner weighted kappa value for all examiners at all angulations was 0.38, range 0.08-0.81, IQR 0.24, Q_1 0.24 and Q_3 0.48. The median inter-examiner percentage agreement for all examiners at all angulations was 77%, range 57%-95%, IQR 11%, Q_1 69% and Q_3 80%.

Table 13. Weighted kappa values (upper value) and percentage agreement (lower value) for inter-examiner reproducibility at 7 degree horizontal and 0 degree angulation (n=12 examiners). The weighted kappa values have been colour coded according to their suggested interpretation as described by Landis and Koch (1977); <0 = Poor agreement, 0.01 - 0.20 = Slight agreement, 0.21 – 0.40 = Fair agreement, 0.41 – 0.60 = Moderate agreement, 0.61 – 0.80 = Substantial agreement, 0.81 – 1.00 = Almost perfect agreement

Examiner	2	3	4	5	6	7	8	9	10	11	12
1	0.36 69%	0.36 69%	0.63 83%	0.63 84%	0.65 85%	0.53 78%	0.66 85%	0.59 82%	0.61 82%	0.52 81%	0.60 83%
2		0.89 96%	0.39 70%	0.28 64%	0.28 65%	0.38 74%	0.35 69%	0.26 63%	0.26 66%	0.18 63%	0.31 70%
3			0.39 70%	0.28 64%	0.27 65%	0.39 74%	0.34 69%	0.27 63%	0.28 67%	0.17 63%	0.32 70%
4				0.61 82%	0.59 81%	0.52 77%	0.55 78%	0.50 76%	0.53 78%	0.40 72%	0.49 76%
5					0.65 85%	0.47 75%	0.58 81%	0.52 79%	0.56 80%	0.48 79%	0.55 80%
6						0.48 76%	0.62 83%	0.56 81%	0.56 80%	0.47 79%	0.53 80%
7							0.49 77%	0.41 73%	0.44 74%	0.31 71%	0.45 76%
8								0.60 82%	0.62 83%	0.47 78%	0.54 80%
9									0.53 79%	0.43 77%	0.47 77%
10										0.48 79%	0.56 81%
11											0.48 81%

The median inter-examiner weighted kappa value for all examiners at 7 degree horizontal and 0 degree angulation was 0.48, range 0.17-0.89, IQR 0.19, Q_1 0.37 and Q_3 0.56. The median inter-examiner percentage agreement for all examiners at 7 degree horizontal and 0 degree angulation was 78%, range 63%-96%, IQR 11%, Q_1 70% and Q_3 81%.

Table 14. Weighted kappa values (upper value) and percentage agreement (lower value) for inter-examiner reproducibility at 0 degree angulation (n=12 examiners). The weighted kappa values have been colour coded according to their suggested interpretation as described by Landis and Koch (1977); <0 = Poor agreement, 0.01 - 0.20 = Slight agreement, 0.21 – 0.40 = Fair agreement, 0.41 – 0.60 = Moderate agreement, 0.61 – 0.80 = Substantial agreement, 0.81 – 1.00 = Almost perfect agreement

Examiner	2	3	4	5	6	7	8	9	10	11	12
1	0.45 73%	0.44 73%	0.71 86%	0.71 86%	0.71 86%	0.65 83%	0.79 90%	0.65 83%	0.76 88%	0.55 79%	0.64 83%
2		0.91 96%	0.43 72%	0.34 65%	0.31 65%	0.38 72%	0.38 68%	0.36 68%	0.37 69%	0.22 63%	0.37 70%
3			0.43 72%	0.32 64%	0.30 64%	0.38 72%	0.38 68%	0.34 67%	0.36 69%	0.22 63%	0.36 70%
4				0.64 83%	0.66 84%	0.52 77%	0.67 84%	0.60 81%	0.68 84%	0.45 74%	0.56 79%
5					0.71 86%	0.51 76%	0.70 86%	0.63 82%	0.69 85%	0.48 77%	0.59 81%
6						0.51 77%	0.73 87%	0.62 82%	0.63 83%	0.48 78%	0.58 81%
7							0.60 80%	0.48 75%	0.54 78%	0.40 73%	0.51 77%
8								0.70 86%	0.72 87%	0.52 78%	0.58 80%
9									0.65 83%	0.46 76%	0.52 78%
10										0.56 80%	0.64 83%
11											0.52 80%

The median inter-examiner weighted kappa value for all examiners for 0 degree angulation was 0.53, range 0.22-0.91, IQR 0.24, Q_1 0.41 and Q_3 0.65. The median inter-examiner percentage agreement for all examiners for 0 degree angulation was 79%, range 63%-96%, IQR 11%, Q_1 72% and Q_3 83%.

Part 2: The discriminatory ability and reproducibility of a grading system for assessing observed changes in the proximal relationships of teeth on paired digital radiographs taken with horizontal variations in the X-ray source subject projection geometry

4.6 Diagnostic quality of the digital radiographs

Sixty of the 160 digital radiographs obtained for Part 1 of this research project were used for Part 2. The 20, 0 degree angulation 12 hour post-demineralisation digital radiographs, 20, 7 degree horizontal angulation pre-demineralisation digital radiographs and 20, 15 degree horizontal angulation pre-demineralisation digital radiographs were selected. A random positioning error associated with the PSP plate for the 15 degree horizontal angulation pre-demineralisation digital radiograph for one of the posterior dental arch sextants resulted in the crowns of the teeth not being entirely visible so was therefore not of diagnostic quality. Fifty-nine digital radiographs were of adequate diagnostic quality for use in Part 2 of this research project.

4.7 Demographics of the 13 examiners

The demographics of the 13 independent examiners are displayed in Table 15. Nine of the examiners from Part 1 participated in Part 2 of this research project, in addition to 4 additional examiners who were recruited for Part 2 only.

Table 15. Demographics of the 13 examiners who participated in Part 2

Age (years)	
Median	40
Mean	38.4
Range	25 - 56
Sex	
Male : Female	6(46%) : 7(54%)
Years qualified as a dentist	
Median	15
Mean	15.1
Range	2 - 31
Registered specialist in Dental and Maxillofacial Radiology	
Yes : No	0 : 13
Number of intra-oral radiographs viewed per week (clinical and research)	
Median	20
Mean	17.8
Range	0 - 50
Percentage of intra-oral radiographs viewed that involve the detection and/or monitoring of carious lesions (clinical and research)	
Median	90%
Mean	76%
Range	0% - 100%
Confidence interpreting intra-oral radiographs (0 = not at all confident, 10 = very confident)	
Median	8
Mean	7.8
Range	7 - 9
Confidence using intra-oral radiographs for detecting and/or monitoring carious lesions (0 = not at all confident, 10 = very confident)	
Median	8
Mean	7.5
Range	5 - 9

4.8 Median differences in score for the proximal relationships of teeth on paired digital radiographs following 7 and 15 degree horizontal angulation variations in X-ray source subject projection geometry compared to 0 degree angulation

Table 16 shows the examiners' median differences in scores for the proximal relationship between the 6/7 and between the 5/6 (n=13 examiners), comparing the digital radiographs taken with a 7 degree and 15 degree horizontal angulation to the 0 degree angulation digital radiograph. The combined score when the proximal relationships between all three teeth (5/6/7) are added together is also displayed.

Table 16. Median differences in score for each examiner (n=13) for the proximal relationship between the 6/7 and between the 5/6, in addition to the combined score for 5/6/7, comparing the digital radiographs taken with a 7 degree and 15 degree horizontal angulation to the 0 degree angulation digital radiograph

Examiner	Median differences in score for 5/6 contact for 7 degree horizontal angulation change	Median differences in score for 6/7 contact for 7 degree horizontal angulation change	Median differences in score for 5/6/7 contacts for 7 degree horizontal angulation change	Median differences in score for 5/6 contact for 15 degree horizontal angulation change	Median differences in score for 6/7 contact for 15 degree horizontal angulation change	Median differences in score for 5/6/7 contacts for 15 degree horizontal angulation change
1	0	0.5	0	2	1	2
2	0	1	0	2	1	1.5
3	1	0.5	1	2	1	1
4	1	0	1	2	1	2
5	0.5	1	1	2	1	2
6	0	0.5	0	1	1	1
7	0	1	0	2	1	2
8	0	0	0	2	1	2
9	0	0	0	1	1	1
10	0.5	0.5	0.5	2	1	2
11	1	1	1	2	1	2
12	0	0.5	0	2	1	1.5
13	0	1	0	2	1	1.5
All 13 examiners	0	1	0	2	1	2

4.9 Assessment of intra-examiner reproducibility of the grading system

The weighted kappa values and percentage agreement are presented in Table 17. The median intra-examiner weighted kappa value was 0.86, range 0.79-0.94, IQR 0.06, Q_1 0.84 and Q_3 0.90. The median percentage agreement was 97%, range 95%-99%, IQR 2%, Q_1 96% and Q_3 98%.

Table 17. Weighted kappa values and percentage agreement for intra-examiner reproducibility (n=13 examiners). The weighted kappa values have been colour coded according to their suggested interpretation as described by Landis and Koch (1977); <0 = Poor agreement, 0.01 - 0.20 = Slight agreement, 0.21 – 0.40 = Fair agreement, 0.41 – 0.60 = Moderate agreement, 0.61 – 0.80 = Substantial agreement, 0.81 – 1.00 = Almost perfect agreement

Examiner	1	2	3	4	5	6	7	8	9	10	11	12	13
Weighted Kappa	0.94	0.83	0.82	0.90	0.86	0.86	0.84	0.91	0.87	0.91	0.85	0.90	0.79
Percentage Agreement	99%	96%	96%	98%	96%	97%	97%	98%	97%	98%	96%	98%	95%

4.10 Assessment of inter-examiner reproducibility of the grading system

The weighted kappa values and percentage agreement are presented in Table 18. The median inter-examiner weighted kappa value was 0.83, range 0.71-0.93, IQR 0.07, Q_1 0.80 and Q_3 0.87. The median percentage agreement was 96%, range 93%-98%, IQR 2%, Q_1 95% and Q_3 97%.

Table 18. Weighted kappa values (upper value) and percentage agreement (lower value) for inter-examiner reproducibility (n=13 examiners). The weighted kappa values have been colour coded according to their suggested interpretation as described by Landis and Koch (1977); <0 = Poor agreement, 0.01 - 0.20 = Slight agreement, 0.21 – 0.40 = Fair agreement, 0.41 – 0.60 = Moderate agreement, 0.61 – 0.80 = Substantial agreement, 0.81 – 1.00 = Almost perfect agreement

Examiner	2	3	4	5	6	7	8	9	10	11	12	13
1	0.80 95%	0.82 96%	0.85 97%	0.89 97%	0.83 96%	0.91 98%	0.93 98%	0.88 97%	0.85 97%	0.80 95%	0.86 97%	0.87 97%
2		0.71 93%	0.74 94%	0.81 95%	0.84 96%	0.76 94%	0.78 95%	0.72 94%	0.71 93%	0.75 94%	0.82 96%	0.76 95%
3			0.90 98%	0.78 95%	0.80 95%	0.87 97%	0.84 96%	0.87 97%	0.87 97%	0.81 95%	0.80 95%	0.82 96%
4				0.80 95%	0.78 95%	0.90 98%	0.87 97%	0.85 97%	0.89 98%	0.81 95%	0.84 96%	0.83 96%
5					0.85 97%	0.85 97%	0.86 97%	0.79 95%	0.77 95%	0.80 95%	0.88 97%	0.84 97%
6						0.84 96%	0.80 95%	0.77 95%	0.76 95%	0.73 94%	0.83 96%	0.82 96%
7							0.89 98%	0.87 97%	0.88 97%	0.82 96%	0.87 97%	0.85 97%
8								0.89 98%	0.84 96%	0.82 95%	0.87 97%	0.85 97%
9									0.86 97%	0.81 95%	0.81 96%	0.87 97%
10										0.82 95%	0.81 95%	0.84 96%
11											0.82 95%	0.78 95%
12												0.80 96%

5 Discussion

This thesis was carried out in two parts. Part 1 assessed *in-vitro* the accuracy and reproducibility of DSR for detecting demineralisation in occlusal cavities using digital radiographs taken with variations in X-ray source subject projection geometry after varying time periods of demineralisation. It found that the highest accuracy was achieved when digital subtraction images were produced using digital radiographs that had been taken with a reproducible 0 degree X-ray projection geometry after the longest period of demineralisation, 24 hours. If a 7 degree horizontal angulation variation in X-ray source subject projection geometry existed between the digital radiographs used to produce the digital subtraction images, no statistically significant reduction in accuracy was detected following 12 and 24 hours demineralisation. The intra- and inter-examiner reproducibility of DSR for detecting demineralisation in occlusal cavities under these circumstances was moderate.

Part 2 investigated the discriminatory ability and evaluated the reproducibility of a grading system for assessing observed changes in the proximal relationships of teeth on paired digital radiographs that had been taken with horizontal variations in X-ray source subject projection geometry. It found that when a 7 degree horizontal angulation variation in X-ray source subject projection geometry existed between paired digital radiographs, the majority of differences in scores were less than 1. In the majority of cases this represented observed differences in the size of inter-proximal spacing or proximal overlapping between teeth less than half the width of enamel. However, when a 15 degree horizontal angulation variation in X-ray source subject projection geometry existed between paired digital radiographs, the majority of differences in scores were 1 or more. Irrespective of the size of horizontal angulation variation in X-ray source subject projection geometry that existed between paired digital radiographs, the intra- and inter-examiner reproducibility of the grading system was almost perfect. The ability of the grading system to identify the extent of horizontal angulation variations in X-ray source subject projection geometry that may exist between paired digital radiographs provides useful information to indicate their suitability to undergo digital subtraction and produce images that have a high diagnostic accuracy.

Part 1: *In-vitro* assessment of the accuracy and reproducibility of DSR for detecting demineralisation in occlusal cavities using digital radiographs taken with variations in X-ray source subject projection geometry after varying time periods of demineralisation

5.1 Limitations associated with the methodology

The 160 digital radiographs used in Part 1 of this research project were originally produced for a different research project with 80 used in the study by Ricketts *et al.* (2007). The sample size of 40 molar teeth, variations in X-ray source subject projection geometry and length of demineralisation were pre-established and could not be altered.

No power calculation was carried out for the study by Ricketts *et al.* (2007) to identify the sample size. Instead a convenience sample of 40 molar teeth was used, which demonstrated that after 12 hours demineralisation or longer, DSR was statistically significantly more accurate and reproducible ($P<0.01$) compared to viewing paired digital radiographic images side by side for identifying demineralisation in occlusal cavities when a reproducible X-ray projection geometry was used at 0 degree angulation (Ricketts et al., 2007). For these reasons, no power calculation was carried out (nor was it possible) in this research project, and only the digital radiographs obtained after 12, 18 and 24 hours demineralisation were used.

To reduce the number of digital radiographs to be taken, the ones demonstrating variations in X-ray source subject projection geometry (7 and 15 degree horizontal angulation, 10 and 15 degree vertical angulation) compared to 0 degree angulation were taken prior to demineralisation, rather than at each time period following demineralisation. This had no effect on the resulting digital subtraction images.

The digital radiographs of the upper posterior dental arch sextants were taken with the plaster block positioned on the laminated board to ensure reproducible positioning and control of the X-ray projection geometry. To avoid the teeth being oriented upside down compared to the normal clinical orientation, the image was rotated to the correct anatomical orientation during image processing.

The 0 degree angulation digital radiograph was taken with the X-ray beam passing tangentially through the inter-proximal space and/or contact points of the molar and premolar teeth, parallel to the occlusal plane of the teeth. The perspex block, plaster block and PSP plate were positioned parallel to each other and perpendicular to the X-ray beam. As this X-ray projection geometry produces the ‘perfect’ radiographic image which is the standard that bitewing radiographs taken in the clinical setting attempt to reproduce, any variations in X-ray source subject projection geometry used this as their reference.

Although the digital subtraction images were produced by the principal researcher, SR, as the process involved human decision making, small variations in image processing could have occurred during their production. The process for producing the digital subtraction images was therefore standardised as far as possible to reduce the likelihood of variations occurring that could affect their relative diagnostic quality.

Importing the digital subtraction images into Microsoft PowerPoint presentations may have degraded the images in some way, for example by compressing the image size. The digital subtraction images were however acceptable for diagnostic purposes and subjectively no differences were detectable between the Microsoft PowerPoint images and the original .bmp files. The monitors and viewing conditions were also not standardised between examiners, however, the conditions used by each examiner reflected their clinical practice and were acceptable. Any differences would represent the variability that would be representative of that found in the clinical setting.

Twelve of the 600 digital subtraction images were not of diagnostic quality to enable assessment of demineralisation in the occlusal cavities, so only 588 digital subtraction images were available for use in Part 1. Although this reduced the number of available digital subtraction images, in particular the proportion of digital subtraction images produced with a 15 degree horizontal angulation variation in X-ray source subject projection geometry, it will have had negligible effect on the overall results which were statistically significant.

Although there was a random selection process used in producing the viewing order of the initial 588 and repeat 100 digital subtraction images, they were the same for all 12 examiners. Growing examiner experience and confidence could have favoured image interpretation towards the later parts of the presentations. Examiner fatigue could have however resulted in the opposite effect occurring, favouring the interpretation of the digital subtraction images towards the earlier parts of the presentation. To reduce fatigue, the examiners were advised to only view 100 digital subtraction images at a time, taking around 30 minutes, over 6 sessions. These possible systematic effects or errors could have been reduced if the order of the subtraction images had been randomised for each individual examiner.

For intra-examiner reproducibility, 100 of the initial 588 digital subtraction images, were randomly selected for independent re-examination by the 12 examiners, who were kept blind to previous results. Although no statistical analysis was carried out to assess if this sample size would provide a representative sample to enable accurate assessment of intra-examiner reproducibility, it was similar to the proportion used in the study by Ricketts *et al.* (2007). The images were viewed one month after their first viewing to ensure adequate washout time, preventing the examiners from recognising them and remembering their previous score. It could be argued that the examiners would have gained greater experience, and possible confidence having viewed and scored the initial 588 digital subtraction images prior to viewing and scoring the repeat 100 digital subtraction images which could have favoured the interpretation of the repeat 100 digital subtraction images. The extent of this effect or error could have been reduced if the 100 randomly selected repeat images were inserted into the original Microsoft PowerPoint presentations of the initial 588 images. However, in this situation there could be an increased chance that the examiners may recognise repeat images and the score allocated.

Although a random selection process was used to assess intra-examiner reproducibility, it did not ensure that an equal proportion of digital subtraction images were selected to represent each variation in X-ray projection geometry and length of demineralisation. To include stratification and ensure that equal proportions were represented in the repeat scorings, there would have to have been a much larger number of images to re-score, possibly resulting in increased subject fatigue and participant dropout. The reproducibility values obtained therefore reflected the overall intra-examiner reproducibility when interpreting digital subtraction images.

5.2 Interpretation of the results – Accuracy of DSR for detecting demineralisation in occlusal cavities

5.2.1 Interaction between variations in X-ray source subject projection geometry and length of demineralisation

Univariate analysis of variance demonstrated a statistically significant interaction ($F=2.778$, $P<0.01$) between variations in X-ray source subject projection geometry and length of demineralisation regarding the accuracy of DSR for detecting demineralisation in occlusal cavities. For this reason, the effect that variations in X-ray source subject projection geometry had on the accuracy of DSR for detecting demineralisation in occlusal cavities were analysed at each specific length of demineralisation.

5.2.2 Variation in X-ray source subject projection geometry

One-way analysis of variance demonstrated that variations in X-ray source subject projection geometry had a statistically significant effect ($P<0.001$) on the accuracy of DSR for detecting demineralisation in occlusal cavities after 12 hours, 18 hours and 24 hours demineralisation. This finding agrees with the literature where reliable DSR requires the two digital radiographic images used to produce the subtraction image to be taken with reproducible X-ray projection geometry (Christgau et al., 1998, Dove and McDavid, 1992, Eberhard et al., 2000, Haiter-Neto et al., 2005, Janssen and van Aken, 1989, Rudolph et al., 1987, Ruttimann et al., 1981, van der Stelt, 1993, van der Stelt, 2008, Wenzel, 2002, Wenzel et al., 1993, Hausmann et al., 1991).

Post-hoc tests for multiple comparisons using a Bonferroni correction to reduce the chance of obtaining a type I error identified that after 12 and 24 hours demineralisation, there was no statistically significant reduction ($P=1.000$) in the accuracy of DSR for detecting demineralisation in occlusal cavities using digital radiographs that had a 7 degree horizontal angulation variation in X-ray source subject projection geometry between them, compared to the use of a reproducible 0 degree X-ray projection geometry. However, this pattern was not identified after 18 hours demineralisation as a statistically significant reduction ($P<0.001$) in accuracy was detected. The reason for this anomaly is not clear but it could have been a spurious finding. At all extents of demineralisation, any vertical angulation variation or 15 degree horizontal angulation variation in X-ray source subject projection geometry between digital radiographs resulted in a statistically significant reduction ($P<0.001$) in the accuracy of DSR for detecting demineralisation in occlusal cavities compared to the use of a reproducible 0 degree X-ray projection geometry.

Clinically, it is important to identify demineralisation in occlusal cavities as early as possible so that additional preventative measures can be put in place to reduce the extent of tooth tissue destruction. This research project demonstrates that after 12 hours artificial demineralisation, although the highest accuracy with DSR was obtained using digital radiographs that had been taken with a reproducible 0 degree angulation X-ray projection geometry (AuROC curve = 0.858), no statistically significant reduction ($P=1.000$) in accuracy was detected if digital radiographs have a 7 degree horizontal angulation variation between them (AuROC curve = 0.826). Paired samples t-test also demonstrated that after all extents of demineralisation, although there was a trend for higher accuracy favouring a distal shift in X-ray source, no statistically significant difference ($P\geq0.05$) in accuracy was detected if the 7 degree horizontal angulation variation was either in a mesial or distal direction.

A 7 degree horizontal angulation variation in X-ray source subject projection geometry between digital radiographs is greater than the 3 degrees of variation which can be controlled for, over a 6 month period, using customised bite blocks with commercially available devices (Duckworth et al., 1983, Rudolph and White, 1988). However, further research is needed to assess if a 5 degree vertical angulation variation in X-ray source subject projection geometry between digital radiographs compared to the use of a reproducible 0 degree angulation X-ray projection geometry for the production of a digital subtraction image would statistically significantly reduce its accuracy for detecting demineralisation in occlusal cavities. This is required as a 5 degree vertical angulation variation in X-ray source subject projection geometry between digital radiographs is the extent of variation which can be controlled for, over a 6 month period, using customised bite blocks with commercially available devices (Duckworth et al., 1983, Rudolph and White, 1988). This research project demonstrated that if digital radiographs with either a 10 or 15 degree vertical angulation variation in X-ray source subject projection geometry between them were used to produce a digital subtraction image, that its accuracy for detecting demineralisation in occlusal cavities was statistically significantly reduced ($P<0.001$) compared to the use of digital radiographs with a reproducible 0 degree X-ray projection geometry.

This research project also found that, for occlusal cavities, as the size of the angulation variation of the X-ray source subject projection geometry between digital radiographs to produce a digital subtraction image increased, both horizontally and vertically, then the corresponding accuracy for detecting demineralisation decreased for all extents of demineralisation. This inversely proportional relationship between the increasing size of angulation variation, and, its reduced accuracy agrees with other findings (Davis et al., 1994, Grondahl et al., 1984, Rudolph et al., 1987, Wenzel, 1989).

5.2.3 Length of demineralisation

As the extent of demineralisation increased, so did the accuracy of DSR for detecting demineralisation in occlusal cavities using a reproducible 0 degree X-ray projection geometry. However, following a 7 degree horizontal angulation variation in X-ray source subject projection geometry between the digital radiographs used to produce a digital subtraction image, although the accuracy following 24 hours demineralisation was higher than that after 12 hours, the accuracy after 18 hours was lower than that after 12 hours. There is no obvious reason for this anomaly. When a reproducible 0 degree X-ray projection geometry was used, this research project reported slightly lower mean AuROC curves for all 12 examiners (mean AuROC curve after 12 hours demineralisation = 0.858, mean AuROC curve after 18 hours demineralisation = 0.924, mean AuROC curve after 24 hours demineralisation = 0.946) compared to those calculated for all 5 examiners in the study by Ricketts *et al.* (2007) (mean AuROC curve after 12 hours demineralisation = 0.952, mean AuROC curve after 18 hours demineralisation = 0.976, mean AuROC curve after 24 hours demineralisation = 0.956). Although this project and the Ricketts *et al.*

(2007) study used the same digital radiographs, different individuals manipulated them to produce the digital subtraction images. Small procedural variations related to human decision making during the production of the images could explain this, however, the most likely explanation for why this study's reported accuracy was slightly lower than Ricketts *et al*. (2007) would be examiner experience and confidence interpreting digital subtraction images as 4 out of the 5 examiners (80%) in the 2007 study had experience of viewing digital subtraction images, however, in this research project, only six out of the twelve examiners (50%) had any experience of DSR.

5.2.4 Comparison with other studies

Two *in-vitro* studies investigated the accuracy of DSR for detecting acid induced demineralisation at proximal enamel surfaces of teeth (Haiter-Neto et al., 2005, Ferreira et al., 2006). Both used a reproducible X-ray projection geometry to produce logarithmically contrast enhanced digital subtraction images, and the AuROC curve for the detection of demineralisation within the proximal surfaces of enamel was reported as 0.98 in both studies. It is difficult to directly compare these results with this research project and Ricketts *et al.* (2007), as each used a different demineralising protocol and the degree of demineralisation may very well vary, even under strictly controlled timeframes. However, the results of Ferreira *et al.* (2006) and Haiter-Neto *et al.* (2005) appear to suggest that DSR is more accurate for detecting acid induced demineralisation in proximal enamel surfaces compared to occlusal surfaces when a reproducible X-ray projection geometry has been used. This would be logical considering the anatomy at both sites and the different amount of sound buccal and lingual enamel and dentine which would attenuate the X-ray beam.

However, the accuracy for detecting demineralisation in proximal sites using DSR may be significantly reduced if digital radiographs are used that have variations in horizontal angulation of the X-ray source subject projection geometry resulting in superimposition of the proximal surfaces of the teeth. It has been demonstrated that even when Rinn loops No.2 ® and Kwik-Bite filmholders ® are used to obtain bitewing radiographs of unrestored permanent teeth that have proximal contact points, that only 19% of the surfaces show no overlapping (Sewerin, 1981a). It has also been long known that variations in horizontal X-ray source subject project geometry can cause artifactual radiographic changes on conventional radiographs regarding the apparent depth of proximal radiolucencies associated with carious lesions (Haugejorden, 1974, Benn and Watson, 1989, van der Stelt et al., 1989, Sewerin, 1981b) and as the size of angulation of variation of the X-ray source increases, so does the apparent depth (Chadwick et al., 1999).

5.3 Interpretation of the results – Reproducibility of DSR for detecting demineralisation in occlusal cavities

5.3.1 Intra-examiner reproducibility

Although the median percentage agreement for intra-examiner reproducibility for the 12 examiners was high at 86%, percentage agreement does not take into account agreement occurring by chance. Kappa calculations were carried out as these take into account the amount of agreement that would be expected by chance, and by assigning weights, also, the degree and importance of disagreements. The median weighted kappa value for the 12 examiners was 0.585, which using the interpretation published by Landis and Koch (1977) represents moderate agreement. In this research project linear weights were used, which by definition were proportional to the five-point certitude scale.

The study by Ricketts *et al.* (2007), using the same digital radiographs as this research project taken with a reproducible 0 degree angulation X-ray source subject projection geometry, calculated the mean intra-examiner reproducibility kappa value for the five examiners at each length of demineralisation. However, due to a small sample size no kappa value was calculable for 12 hours. The mean intra-examiner reproducibility kappa value for the five examiners in the Ricketts *et al.* (2007) study after 18 hours demineralisation was 0.52 and after 24 hours demineralisation was 0.61, which using the interpretation published by Landis and Koch (1977) represents moderate agreement, the same as was identified in this research project. This research project involved variations in X-ray source subject projection geometry which the Ricketts *et al.* (2007) study did not. This suggests that the use of DSR for detecting demineralisation in occlusal cavities has moderate intra-examiner reproducibility, even when digital subtraction images are produced with variations in X-ray source subject projection geometry.

Four *in-vivo* studies by Martignon *et al.* (2006), Martignon *et al.* (2012), *Paris et al.* (2010) and Wenzel *et al.* (2000) (discussed in the literature review), investigated and compared the qualitative analysis of DSR with other conventional radiographic methods for assessing the progression of carious lesions in both enamel and dentine. Three reported intra-examiner kappa values for DSR when a reproducible X-ray projection geometry had been used. The study by Wenzel *et al.* (2000) investigated lesions on all surfaces and reported an intra-examiner reproducibility kappa value for DSR of 0.875. The studies by Martignon *et al.* in 2006 and 2012 investigated proximal surfaces only and reported intra-examiner reproducibility kappa values for DSR of 0.87 and 0.78 respectively. These three studies all reported higher intra-examiner reproducibility kappa values than this research project found, however, this could be explained by the scoring systems used to assess carious lesion behaviour and the way intra-examiner reproducibility was calculated (Martignon et al., 2006, Martignon et al., 2012, Wenzel et al., 2000). In this project a five-point certitude scale was used, and all five codes were used when calculating intra-examiner reproducibility. However, in the study by Wenzel *et al.* (2000), although the scoring system comprised of five codes, when these codes were used to assess reproducibility, they were grouped to create dichotomous data as either ‘no change’ or ‘change’ in surface appearance of the carious lesion. In the study by Martignon *et al.* (2006) the scoring system had three codes that were used to assess reproducibility, either ‘progression’, ‘no changes’ or ‘regression’, and in the other study by Martignon *et al.* (2012) the scoring system had two codes that were used to assess reproducibility, either ‘stabilised’ or ‘progressed’. The use of fewer codes in the scoring systems and dichotomisation of data in these studies is likely to have resulted in higher intra-examiner kappa values. This effect was demonstrated by Ricketts *et al.* (2007) who used the same five-point certitude scale that was used in this research project. They showed that if intra-examiner reproducibility kappa values were calculated by grouping the scores into two groups, no demineralisation (score 1, 2 and 3) and demineralisation (score 4 and 5), that this resulted in higher kappa values as the mean intra-examiner reproducibility kappa score after 12, 18 and 24 hours demineralisation combined was 0.94, rather than 0.52 and 0.61 after 18 and 24 hours demineralisation respectively, if all five codes were used.

Whilst comparisons have been made in the aforementioned text, it has however been suggested that it is not appropriate to compare kappa values for reproducibility from different studies investigating carious lesion diagnosis, as differences between study design, examiners' experience and the number of codes in the scoring systems used all influence the outcome (Poulsen et al., 1980, Mileman et al., 1983, Pliskin et al., 1984, Espelid and Tveit, 1986, Naitoh et al., 1998). Indeed, these variables may also account for, or contribute to, the differences cited above between studies.

5.3.2 Inter-examiner reproducibility

Inter-examiner reproducibility was calculated for digital subtraction images that had been produced using all variations in X-ray source subject projection geometry combined. It was also calculated for 0 degree angulation only as this angulation resulted in the highest accuracy for detecting demineralisation in occlusal cavities using DSR and also enabled comparison with the results from the study by Ricketts *et al.* (2007). Further calculations were also carried out combining 0 degree and 7 degree horizontal angulations as this research project demonstrated that a 7 degree horizontal angulation variation in X-ray source subject projection geometry compared to 0 degrees resulted in no statistically significant reduction in accuracy for detecting demineralisation in occlusal cavities using DSR after 12 and 24 hours demineralisation.

The median inter-examiner weighted kappa value for the 12 examiners for 0 degree angulation only was 0.53, which using the Landis and Koch (1977) interpretation, represents moderate agreement. This value is slightly higher than the mean inter-examiner reproducibility kappa values reported for the five examiners in the Ricketts *et al.* (2007) study, using the same digital radiographs as this research project, taken with 0 degree angulation after 12 hours (mean kappa value = 0.473), 18 hours (mean kappa value = 0.471) and 24 hours (mean kappa value = 0.484) demineralisation. Despite this small difference, both this research project and the study by Ricketts *et al.* (2007) demonstrated that DSR had moderate inter-examiner reproducibility for detecting demineralisation in occlusal cavities when a reproducible X-ray projection geometry has been used with 0 degree angulation digital radiographs.

Two *in-vivo* studies previously mentioned also reported inter-examiner reproducibility when a reproducible X-ray projection geometry had been used (Paris et al., 2010, Wenzel et al., 2000). Wenzel *et al.* (2000) investigated lesions on all surfaces and reported an inter-examiner reproducibility kappa value for DSR of 0.678. The study by Paris *et al.* (2010) investigated proximal surfaces only and reported an inter-examiner reproducibility kappa value for DSR of 0.809. The scoring system in the Paris *et al.* (2010) study used three codes to assess carious lesion behaviour as either ‘progression’, ‘regression’ or ‘stable lesion’. Both studies reported higher inter-examiner reproducibility kappa values than identified in this research project, however, this is likely to be due to reasons previously alluded to regarding the scoring systems that were used to assess carious lesion behaviour and the way inter-examiner reproducibility was calculated by grouping codes together (Paris et al., 2010, Wenzel et al., 2000). The heterogeneity between the studies also makes direct meaningful comparisons difficult, if not impossible (Poulsen et al., 1980, Mileman et al., 1983, Pliskin et al., 1984, Espelid and Tveit, 1986, Naitoh et al., 1998).

When 0 degree and 7 degree horizontal angulation were combined, this research project demonstrated that the median inter-examiner weighted kappa value for all 12 examiners was 0.48, which although slightly lower than that reported for 0 degree angulation only, still represents moderate agreement using the interpretation published by Landis and Koch (1977). A notable reduction in inter-examiner reproducibility was however identified when all variations in X-ray source subject projection geometry were combined as the median weighted kappa value for all 12 examiners was 0.38, representing only a fair agreement (Landis and Koch, 1977). However, this is irrelevant as this research project demonstrated a statistically significant reduction in accuracy for detecting demineralisation in occlusal cavities using DSR when 15 degree horizontal, 10 degree vertical and 15 degree vertical variations in X-ray source subject projection geometry existed between digital radiographs compared to a 0 degree angulation reproducible X-ray projection geometry.

Part 2: The discriminatory ability and reproducibility of a grading system for assessing observed changes in the proximal relationships of teeth on paired digital radiographs taken with horizontal variations in the X-ray source subject projection geometry

5.4 Limitations associated with the methodology

The 60 digital radiographs used in Part 2 of this research project were produced by a group of researchers, 20 of which were used in the study by Ricketts *et al.* (2007). The sample size of 20 premolar teeth and 40 molar teeth, and the anatomical relationship between the one premolar tooth and two molar teeth in each of the 20 posterior dental arch sextants was therefore pre-determined. Although there was a range in the size of inter-proximal spacing between teeth, none of the posterior dental arch sextants simulated crowding which can be present in the clinical setting. The 7 degree and 15 degree horizontal angulation variations in X-ray source subject projection geometry compared to 0 degree used to produce the digital radiographs were also pre-determined as previously mentioned in section 5.1.

Due to a random positioning error associated with the PSP plate for the 15 degree horizontal angulation digital radiograph for one of the posterior dental arch sextants, only 59 digital radiographs, rather than 60, were of adequate diagnostic quality for use in Part 2 of this research project. Although this reduced the proportion of digital radiographs demonstrating a 15 degree horizontal angulation, it will have had minimal or negligible effect on the overall results.

Following a literature search, the principal researcher (SR) was unable to identify an existing ordinal categorical grading system designed to assess the observed changes in the proximal relationships of teeth on paired (digital) radiographic images that correlates with variations in horizontal angulation X-ray source subject projection geometry. The grading system used in this research project was therefore designed for the needs of the project and has not been described or validated. Although a number of studies have investigated the degree of proximal overlapping on radiographs, very few specify exactly what criteria have been used to assess it (Pitts, 1984). However, the section of the grading system used to assess the degree of proximal overlap is very similar to criteria described by Sewerin (1981a), McDonald (1983) and Pitts (1984). One limitation associated with the grading system is that the scores are based on the ‘width of enamel’ as a reference for measuring both the extent of interproximal spacing and proximal overlapping. The width of enamel varies between teeth, and is not easily assessed if teeth have proximal restorations in place.

Although the order of the digital radiographs for the initial 60 and reproducibility presentations was randomly selected for the two Microsoft PowerPoint presentations, they were the same for all 13 examiners. Examiner fatigue could have favoured the scoring of the digital radiographic images towards the earlier parts of the presentation. The extent that this systematic effect or error could have had might have been reduced if the order of the digital radiographic images had been individually determined by a random selection process for each of the 13 examiners’ presentations.

As previously mentioned in section 5.1, the digital radiographs of the upper posterior dental arch sextants were taken with the plaster block positioned on the laminated board to ensure reproducible positioning and control of the X-ray projection geometry. To avoid the teeth being oriented upside down compared to the normal clinical orientation the image was rotated to the correct anatomical orientation during image processing. Importing the digital radiographic images into Microsoft PowerPoint presentations may have also degraded the images in some way, for example by compressing the image size. The digital radiographic images were however acceptable for diagnostic purposes and subjectively no differences could be identified comparing the images in the Microsoft PowerPoint presentations to the images saved as .bmp files. The monitors and viewing conditions were also not standardised between examiners, however, the conditions used by each examiner reflected their clinical practice and were acceptable. Any differences would represent the variability that would be representative of that found in the clinical setting.

5.5 Interpretation of the results

5.5.1 Assessment of the median differences in score for the proximal relationships of teeth on paired digital radiographs following 7 and 15 degree horizontal angulation variations in X-ray source subject projection geometry compared to 0 degree angulation

Following a 7 degree horizontal angulation variation in X-ray source subject projection geometry between paired digital radiographs, the majority of examiners reported median differences in score for the proximal relationships between teeth of less than 1. In the majority of cases this represented observed differences in the size of inter-proximal spacing or proximal overlapping between teeth less than half the width of enamel. However, following a 15 degree horizontal angulation variation in X-ray source subject projection geometry, the majority of examiners reported median differences in score of 1 or more. Part 1 of this research project demonstrated that the accuracy for detecting demineralisation in occlusal cavities was not statistically significantly reduced following a 7 degree horizontal angulation variation in X-ray source subject projection geometry compared to the use of a reproducible 0 degree X-ray projection geometry, however, it was following a 15 degree horizontal angulation variation. The grading system could therefore be used to assess the paired digital radiographs used in this research project, and aid identification of the extent of horizontal angulation variations in X-ray source subject projection geometry that existed between them. This useful information would then enable a decision to be made as to their suitability to undergo digital subtraction and the resulting accuracy of the digital subtraction image for detecting demineralisation in occlusal cavities.

Following a 7 degree horizontal angulation variation in X-ray source subject projection geometry, the largest median differences in score were identified for the proximal relationship between the 6/7 contact, rather than the 5/6 contact, however, the opposite was identified following a 15 degree horizontal angulation variation. Clinically, as the width of the contact points between teeth are usually wider in the buccal-lingual/palatal direction for the 6/7 contact compared to the 5/6 contact, you would expect to see greater proximal overlapping, and therefore a greater difference in scores for the 6/7 contact. The conflicting findings in this *in-vitro* research project are likely related to the positioning of the teeth within the plaster blocks, their anatomical relationship to one another and that the extracted teeth came from different individuals.

To enable identification of the range of horizontal angulation variations in X-ray source subject projection geometry that produce specific scores, further research using the grading system would be required. This may however demonstrate that the grading system used in this research project lacks the discriminatory power to identify smaller differences in horizontal angulation variation, especially increments of 1 degree. It is likely that this would only be achievable using a quantitative continuous grading system, as it has already been demonstrated that a linear relationship exists between the size of variation of X-ray source subject projection geometry and the change in width of proximal overlap observed on paired radiographs (McDonald, 1983). If a 0.1mm increase in width of proximal overlap is detected between paired radiographs, then 95% of the deviations in X-ray source subject projection geometry are less than 2.5 degrees (McDonald, 1983).

5.5.2 Reproducibility of the grading system

5.5.2.1 Intra-examiner reproducibility

The grading system used in this research project had excellent intra-examiner reproducibility, as the median weighted kappa value for intra-examiner reproducibility for the 13 examiners was 0.86, which using the interpretation published by Landis and Koch (1977) represents almost perfect agreement. Only one examiner did not demonstrate almost perfect agreement, however, their weighted kappa value was only 0.02 lower than the threshold value for classifying almost perfect agreement (Landis and Koch, 1977).

As the grading system used in this research project has not been used in any other studies, direct comparisons cannot be made. However, aspects of the grading system used to assess the degree of proximal overlap are very similar and mirror aspects of criteria that have been described in studies by Sewerin (1981a) and McDonald (1983). Unfortunately the study by Sewerin (1981a) did not report intra-examiner reproducibility, however, the study by McDonald (1983) demonstrated 92% concordance of the overlap scores which is similar, albeit slightly lower than the 98% median percentage agreement identified in this research project.

5.5.2.2 Inter-examiner reproducibility

The grading system used in this research project had excellent inter-examiner reproducibility as the median weighted kappa value for inter-examiner reproducibility for the 13 examiners was 0.83, which represents almost perfect agreement (Landis and Koch, 1977). Unfortunately only one examiner assessed the degree of proximal overlap in the studies by Sewerin (1981a) and McDonald (1983) so it is not possible to compare the inter-examiner reproducibility reported in this research project with these studies.

5.6 Limitations of the *in-vitro* model used in this research project compared to the clinical environment

In this research project, the position of the individual teeth in the plaster blocks, and therefore, their relationship to one another remained static to ensure strict control over all possible variables. This differs from the clinical situation, as over time the individual position and relationship of teeth to one another can alter, especially in the developing dentition. This would have implications on the ability to standardise X-ray projection geometry between digital radiographs and affect the quality of any digital subtraction images produced, which has to be respected if the results of this research project are to be applied to the clinical environment. The DSR software can however account for some tooth movement through patch minimisation processes as previously described.

The application of the demineralising solution to the mechanically prepared and modified occlusal cavities of the extracted molar teeth resulted in demineralisation of the dental tissues. However, this is an artificial process compared to the natural disease process where there is a continual process of demineralisation and remineralisation over a much longer period of time involving cariogenic bacteria, various ions that are present in saliva and possibly topical fluoride. It is also not known what net or percentage loss of tooth tissue occurred in this research project following 12, 18 and 24 hours application of the demineralising solution, and how this would correlate to the changes seen over time in a naturally progressing carious lesion. The standardisation of the demineralisation process used in this study was however necessary to ensure strict control over all possible variables.

In the clinical environment, other changes to coronal tooth tissue may occur over time not just as a result of demineralisation of the cavity in question, but due to the development of other carious lesions at other sites on the same tooth, toothwear, trauma and restorative intervention. The results of this research study therefore have to be taken in context when applied to occlusal cavities that have been restored, received micro-invasive treatment or been subjected to preventative strategies involving the application of topical fluoride as all of these will have altered the tooth tissue between subsequent digital radiographs used to produce a digital subtraction image over time.

Also in the clinical environment, additional information is available that may influence the decision as to whether or not a previously identified carious lesion has demineralised further when looking at a digital subtraction image. Examples would include the identification of new carious lesions in other sites, poor plaque control, confirmation of a highly cariogenic diet, lack of use of topical fluoride and a reduction in the quantity or buffering capacity of saliva.

The reproducible X-ray projection geometry used in this research project involved the 0 degree angulation digital radiograph being taken with the X-ray beam passing tangentially through the contact points of the teeth, parallel to the occlusal plane and perpendicular to the X-ray film, as this X-ray projection geometry produces the ‘perfect’ radiographic image which is the standard that bitewing radiographs taken in the clinical setting attempt to reproduce. All variations in X-ray source subject projection geometry used to produce other digital radiographs were applied with respect to this. It is important to appreciate this relationship if the results of this research project are to be applied to the production of digital subtraction images using digital radiographs taken in the clinical setting. Although the ideal X-ray projection geometry is aspired to, it is not always possible, and it has been reported that proximal overlapping in bitewing radiography of permanent teeth that have proximal contact points, occurs in 81% of sites (Sewerin, 1981a).

6 Conclusions

6.1 Research question

This research project demonstrated that alteration of X-ray source subject projection geometry impacted the accuracy and reproducibility of DSR for detecting demineralisation in artificially created occlusal cavities.

6.2 Part 1

6.2.1 Objective 1

DSR had the highest accuracy for detecting demineralisation in occlusal cavities when a reproducible 0 degree X-ray projection geometry was used, that is with the X-ray beam passing tangentially through the contact points of the teeth, parallel to the occlusal plane and perpendicular to the X-ray film to produce the digital radiographic images. If a 7 degree horizontal angulation variation in X-ray source subject projection geometry existed between digital radiographs used to produce a digital subtraction image, it did not statistically significantly reduce its accuracy compared to the use of a reproducible 0 degree X-ray projection geometry after 12 and 24 hours demineralisation. However, 15 degree horizontal, 10 degree vertical and 15 degree vertical angulation variations in X-ray source subject projection geometry between digital radiographs did statistically significantly reduce the accuracy of DSR compared to the use of a reproducible 0 degree X-ray projection geometry after 12, 18 and 24 hours demineralisation.

6.2.2 Objective 2

No statistically significant difference in accuracy for detecting demineralisation in occlusal cavities using DSR was identified comparing a mesial with a distal shift in X-ray source, following a 7 degree horizontal angulation variation in X-ray source subject projection geometry after 12, 18 and 24 hours demineralisation. Following a 15 degree horizontal angulation variation in X-ray source subject projection geometry, a statistically significantly higher accuracy was identified following a distal shift, compared to a mesial shift in X-ray source after 12 and 24 hours demineralisation. However, this finding is not relevant due to the statistically significant reduction in accuracy associated with a 15 degree horizontal angulation variation in X-ray source subject projection geometry compared to a reproducible 0 degree X-ray projection geometry.

6.2.3 Objective 3

No statistically significant differences in accuracy for detecting demineralisation in occlusal cavities using DSR was identified comparing a positive upward with a negative downward shift in X-ray source, following a 10 degree vertical angulation variation in X-ray source subject projection geometry after 12, 18 or 24 hours demineralisation. The same was found for 15 degree vertical angulation variation in X-ray source subject projection geometry after 12 and 18 hours demineralisation. Following a 15 degree vertical angulation variation in X-ray source subject projection geometry, a statistically significantly higher accuracy was identified following a positive upward shift, compared to a negative downward shift in X-ray source after 24 hours demineralisation. However, the findings related to vertical angulation variations in X-ray source subject projection geometry are not relevant due to the statistically significant reduction in accuracy associated with vertical angulation variations in X-ray source subject projection geometry compared to a reproducible 0 degree X-ray projection geometry.

6.2.4 Objective 4

DSR had moderate intra-examiner reproducibility for detecting demineralisation in occlusal cavities, irrespective of whether or not the subtraction images were produced using a reproducible 0 degree X-ray projection geometry or 7 degree horizontal, 15 degree horizontal, 10 degree vertical or 15 degree vertical angulation variations.

6.2.5 Objective 5

The use of DSR for detecting demineralisation in occlusal cavities had moderate inter-examiner reproducibility when digital radiographs were used to produce the digital subtraction images that had been taken with either a reproducible 0 degree X-ray projection geometry or 7 degree horizontal angulation variation. However, when digital subtraction images were produced using digital radiographs that had been taken with either a 0 degree reproducible X-ray projection geometry or following 7 degree horizontal, 15 degree horizontal, 10 degree vertical or 15 degree vertical angulation variations in X-ray source subject projection geometry, inter-examiner reproducibility reduced and was only fair.

6.3 Part 2

6.3.1 Objective 1

When the grading system was used to score the proximal relationships of teeth on paired digital radiographs taken with a 7 degree horizontal angulation variation in X-ray source subject projection geometry between them, the majority of the differences in scores were less than 1. In the majority of cases this represented observed differences in the size of inter-proximal spacing or proximal overlapping between teeth less than half the width of enamel. However, when it was used to score the proximal relationships of teeth on paired digital radiographs taken with a 15 degree horizontal angulation variation in the X-ray source subject projection geometry between them, the majority of the differences in scores were 1 or more. The scores generated by the grading system could therefore be used to aid identification of the extent of horizontal angulation variations in X-ray source subject projection geometry that existed between the paired digital radiographs used in this research project.

6.3.2 Objective 2

The intra-examiner reproducibility of the grading system for assessing observed changes in the proximal relationships of teeth on paired digital radiographs taken with 7 and 15 degree horizontal angulation variations in the X-ray source subject projection geometry between them was almost perfect.

6.3.3 Objective 3

The inter-examiner reproducibility of the grading system for assessing observed changes in the proximal relationships of teeth on paired digital radiographs taken with 7 and 15 degree horizontal angulation variations in the X-ray source subject projection geometry between them was almost perfect.

7 Future research

This research project identified that a 7 degree horizontal angulation variation in X-ray source subject projection geometry between digital radiographs does not statistically significantly reduce the accuracy of DSR for detecting demineralisation in occlusal cavities compared to a reproducible 0 degree X-ray projection geometry. Further *in-vitro* research is required to identify to what extent vertical angulation variations in X-ray source subject projection geometry can be tolerated without statistically significantly reducing the accuracy of DSR for detecting demineralisation in occlusal cavities compared to the use of a reproducible 0 degree X-ray projection geometry. Once this has been identified, these horizontal and vertical angulation limits in variation of X-ray source subject projection geometry should be investigated regarding the accuracy of DSR for detecting demineralisation in proximal sites. This will ascertain the range of horizontal and vertical angulation variations in X-ray source subject projection geometry that can be tolerated between digital radiographs for producing digital subtraction images that have a high accuracy for detecting demineralisation in occlusal and proximal sites.

Further research will then be required to continue to develop a grading system that has the discriminatory power to identify the size of variations in vertical and horizontal X-ray source subject projection geometry that may exist between paired digital radiographs. This should take into account the tolerances that have been identified as being acceptable for the production of highly accurate digital subtraction images for the detection of demineralisation in occlusal and proximal sites.

In-vivo research should then be carried out using paired digital radiographs that have been obtained in the clinical environment for investigating the use of DSR for detecting demineralisation in occlusal and proximal sites. This should also include the monitoring of carious lesions that have been managed using resin infiltration, sealants and the placement of restorations, in addition to those managed with prevention alone. Comparisons should be made to other methods of monitoring carious lesions such as the pairwise comparison of digital radiographs. The use of currently available commercially produced intra-oral devices for standardising X-ray projection geometry will require further research in the clinical environment to confirm whether or not they can control vertical and horizontal angulation variations in X-ray source subject projection geometry within the range of tolerance required to produce highly accurate digital subtraction images as identified in the *in-vitro* studies.

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9 Appendices

9.1 Appendix I

ACCURACY AND REPRODUCIBILITY OF DIGITAL SUBTRACTION RADIOGRAPHY IN THE ASSESSMENT OF DEMINERALISATION WITHIN OCCLUSAL CAVITIES USING BITEWING RADIOGRAPHS TAKEN AT VARYING HORIZONTAL AND VERTICAL ANGULATIONS

PARTICIPANT INFORMATION SHEET

The changing philosophy in caries management has seen a move away from complete surgical excision to more conservative approaches involving incomplete caries removal and sealing the carious biomass within the tooth. These approaches are based upon the microbiology and histopathology of the lesions and pulp-dentine complex reactions. The aim of this approach is to arrest lesion progression and hence preserve pulpal health. However, once sealed, it can be difficult to assess whether lesion progression has been arrested or not and hence the success of the management technique.

Early research by Handelman *et al.* (Handelman et al., 1976) suggested that fissure sealants could be used to manage carious lesions that were radiographically visible on the occlusal surface using bitewing radiographs at recall to monitor lesion arrest/progression. However, more recent in vitro research by Ricketts *et al.* (Ricketts et al., 2007) has demonstrated that subtraction radiography is more reproducible and accurate in detecting occlusal demineralisation than viewing paired digital radiographic images side by side. In this study, a reproducible X-ray projection geometry was used. Whilst the subtraction radiography program allowed a degree of warping (using digital manipulation) to overcome small differences in sequential images, the degree to which this is possible has not been determined.

We would like to invite you to take part in this in-vitro research project which aims to assess the accuracy and reproducibility of digital subtraction radiography as a method for assessing demineralisation within occlusal cavities using bitewing radiographs taken at varying horizontal and vertical angulations.

What will I have to do if I take part?

Participation in this research project will involve looking at subtraction images and assessing whether or not you think there has been demineralisation of the occlusal cavity within the specified tooth indicated in each image. If you agree to participate in this research project you will be sent a short Word document to help familiarise you with subtraction radiography, in particular regarding the viewing and analysis of subtraction images.

How long will it take?

Six hundred subtraction images have been produced and it would take approximately three hours to look at all the images, however it would be advisable rather than look at all the images in one session to look at a maximum of 100 images at one time which would take approximately 30 minutes. To enable assessment of intra-examiner variability you will also be required to look at one hundred repeat images which have been selected at random from the original six hundred images one month later. If you agree to take part it would be expected that you will provide your results within two months of being given the subtraction images to look at.

What are the possible risks of taking part?

The subtraction images will be provided in a PowerPoint file which can be viewed on any PC. The images should be viewed while you are sitting in a comfortable position with the monitor the correct height and distance from your eyes to prevent strain. To prevent fatigue you should not look at the images for any longer than 30 minutes per session.

Are there any possible benefits?

Participation in this research project will improve your familiarity viewing subtraction images. It is envisaged that the results from this research project will be submitted for publication in an appropriate peer reviewed journal. As you will have been involved with the acquisition of data your name will be included as an author if you provide final approval of the article (following any necessary drafting and revision) for publication.

Do I have to take part?

No, taking part is voluntary. If you would prefer not to take part you do not have to give a reason.

What happens to the information that you provide?

The University of Dundee complies with the Data Protection Act and all information will be treated with the strictest confidence.

Funding

This research project has not received any funding.

Ethics approval

The East of Scotland Research Ethics Service has determined that this research project does not require ethical approval.

Can I get any more information?

If you have any other queries please feel free to contact the research team at the address, or via the telephone number or e-mail address given below:

Research Team

Sam Rollings (David Ricketts, Nicola Innes)

Department of Restorative Dentistry, The University of Aberdeen Dental School and Hospital, Cornhill Road, Aberdeen, AB25 2ZR

07877 907913

samrollings@nhs.net

9.2 Appendix II

Accuracy and reproducibility of digital subtraction radiography in the assessment of demineralization within occlusal cavities using bitewing radiographs taken at varying horizontal and vertical angulations

Page 1: Participant Questionnaire

Introduction

Thank you very much for agreeing to participate in this research project investigating the accuracy and reproducibility of digital subtraction radiography in the assessment of demineralization within occlusal cavities using bitewing radiographs taken at varying horizontal and vertical angulations.

I would be grateful if you could please complete this questionnaire which will provide useful information regarding the demographics of the assessors involved in this research project. It will also reveal the range of experience and confidence within the group that assessors have regarding the interpretation of digital subtraction images and detection of caries.

If you have any questions regarding this participant questionnaire please do not hesitate to contact the research team whose details are below.

Thank you again for agreeing to participate in this research project.

Research Team

Sam Rollings (David Ricketts, Nicola Innes)

Department of Restorative Dentistry, The University of Aberdeen Dental School and Hospital, Cornhill Road, Aberdeen, AB25 2ZRsamrollings@nhs.net(+44) 07877 907913

Page 2: Accessibility & Data Protection

Accessibility

You can customise the text size/colour to meet your individual needs by using the accessibility features of your web browser. Further information on how to do this is available from the BBC 'My Web My Way' site http://www.bbc.co.uk/accessibility

If you require this questionnaire in an alternative format please contact samrollings@nhs.net

Data Protection

All data collected in this questionnaire will be held securely.

Page 3

Questions are **mandatory** unless marked otherwise.

1. **Full name:**

2. **Age in years:**

3. **Sex:**
   - Male
   - Female

4. **How many years have you been qualified as a dentist?**

5. **Where do you work?** *(Name of department and/or institution including full international postal address)*

6. **What is your job title?**

7. **What is your main area/specialty of work/expertise?**

8. **Are you a registered specialist in Dental and Maxillofacial Radiology?**

- Yes
- No

9. **How many intra-oral dental radiographs do you look at on average per week (clinical and *in-vitro*)?**

9.a. **What percentage of these would involve the detection and/or monitoring of carious lesions?**

10. **How confident are you interpreting intra-oral dental radiographs?** *0 = not at all confident, 10 = very confident*

- 0
- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9
- 10

11. How confident are you detecting carious lesions and/or their progression/arrest using intra-oral dental radiographs? *0 = not at all confident, 10 = very confident*

- ○ 0
- ○ 1
- ○ 2
- ○ 3
- ○ 4
- ○ 5
- ○ 6
- ○ 7
- ○ 8
- ○ 9
- ○ 10

12. Have you used digital subtraction radiography or interpreted digital subtraction images before?

- ○ Yes
- ○ No

12.a. If **Yes** to Q12, when have you used it?

- ☐ In-vitro research
- ☐ In-vivo research
- ☐ Routine clinical practice
- ☐ Other

12.a.i. If you selected Other, please specify:

12.b. If **Yes** to Q12, did you use it to detect and/or monitor carious lesions?

- ○ Yes
- ○ No

12.b.i. If **Yes** to Q12b, please give further details.

12.c. If **Yes** to Q12, which digital subtraction software packages have you used?

13. **How confident are you interpreting digital subtraction images?***0 = not at all confident, 10 = very confident*

○ 0	○ 1	○ 2
○ 3	○ 4	○ 5
○ 6	○ 7	○ 8
○ 9	○ 10	

14. **How confident are you detecting carious lesions and/or their progression/arrest using digital subtraction images?***0 = not at all confident, 10 = very confident*

○ 0	○ 1	○ 2
○ 3	○ 4	○ 5
○ 6	○ 7	○ 8
○ 9	○ 10	

Note that once you have clicked on the **Continue** button your answers are submitted and you cannot return to review or amend that page.

Page 4: Final Page

Thank you for completing this questionnaire.

9.3 Appendix III

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ACCURACY AND REPRODUCIBILITY OF DIGITAL SUBTRACTION RADIOGRAPHY IN THE ASSESSMENT OF DEMINERALISATION WITHIN OCCLUSAL CAVITIES USING BITEWING RADIOGRAPHS TAKEN AT VARYING HORIZONTAL AND VERTICAL ANGULATIONS

INTRODUCTION TO SUBTRACTION RADIOGRAPHY

Thank you for agreeing to participate in this research project investigating the accuracy and reproducibility of digital subtraction radiography in the assessment of demineralisation within occlusal cavities using bitewing radiographs taken at varying horizontal and vertical angulations. Although you may already be familiar with the viewing and analysis of subtraction images, this short document aims to help standardise the researchers involved in the project. It provides background information on subtraction radiography and highlights important aspects related to the analysis of the subtraction images in this research project.

Background to subtraction radiography

Subtraction radiography is a computer-aided radiographic analysis tool. Digital radiographs comprise an array of pixels, each having a grey scale value from 0 to 256. Subtraction radiography software allows corresponding pixels from one digital radiographic image of an object, to be superimposed on another digital radiographic image of the same object. This then allows subtraction of the corresponding pixels and results in the production of a subtraction image. If there is no difference between the pixel values in a given area the subtraction image is blank, however where there has been either a reduction or an increase in grey scale value between the corresponding pixels of the two digital radiographic images either a lighter or darker shadow will be obtained once the values have been moved into the midrange of the grey scale by adding 128 grey scale levels. This is a useful tool in dentistry as it allows the net loss and gain of mineralized tissue (bone or tooth tissue for example) over time to be identified. This has particular relevance in assessing whether or not a carious lesion has progressed or arrested, especially when it has been sealed into the tooth and can't be visualised directly.

Production of a subtraction image is related to the ability to reproduce the x-ray projection geometry between sequential digital radiographs. Subtraction radiography programmes can compensate for a degree of variation in x-ray projection geometry by warping the image, however, the extent to which this is possible, and its effect on diagnostic accuracy and reproducibility have not been fully investigated. This research project addresses this gap in knowledge.

Materials and methods of this research project

In a previous study (Ricketts et al., 2007), forty extracted molar teeth with unrestored occlusal surfaces were obtained. The extent of caries ranged from sound surfaces to frank cavitation. Two molar teeth were selected and mounted in plaster blocks alongside one extracted premolar tooth to simulate the anatomical relationship of upper and lower posterior teeth. Twenty posterior sextants were produced; 5 with upper left, 5 with upper right, 5 with lower left and 5 with lower right teeth. The fissures of each molar tooth were opened with a diamond bur to expose the dentine. In the carious teeth, only the enamel over the carious dentine was removed.

Five digital radiographs were taken of each sextant using phosphor digital imaging plates. The phosphor digital imaging plates remained at a set distance and parallel to the plaster blocks at all times. Digital radiographs were then taken, with the angulation of the x-ray beam at 0°, 7° and 15° to the horizontal plane and at -10° and +15° to the vertical plane. The 0° horizontal plane was parallel to a line drawn at right angles to the buccal side of the plaster blocks and phosphor digital imaging plates and the 0° vertical plane set at 0° to the horizontal.

Nineteen out of the forty molar teeth were selected at random for demineralisation and a demineralising solution was dropped into the test cavities and continuously replenished for the duration of the study. After 12, 18 and 24 hours the acid was washed out of the cavities with water and a digital radiograph taken at 0° to the horizontal plane.

The resulting 100 digital radiographs were then manipulated (by Sam Rollings) to produce 600 subtraction images for each of the teeth after 12, 18 and 24 hours of possible demineralization and at each of the five varying angulations from 0° (0°, 7° and 15° to the horizontal and -10° and +15° to the vertical). The subtraction images had 128 grey scale levels added to the images to move the values into the mid range of the grey scale.

These are the subtraction images which you will be viewing and scoring your level of certainty as to whether or not demineralisation of the occlusal cavity has occurred. This will be done using a five-point certitude scale. One-hundred of these subtraction images have been selected at random to be viewed and scored again by each examiner 1 month after the initial 600 subtraction images to allow assessment of intra-examiner reliability.

Analysis of subtraction images used in this research project

Figures 1 and 2 are two digital radiographs taken of the same sextant at 0° at baseline and after 24 hours of possible demineralisation respectively.

Figure 1. Digital radiograph, 0° at baseline

Figure 2. Digital radiograph, 0° after 24 hours

If these images are then manipulated using the subtraction radiography software as described above, the following subtraction image is produced (Figure 3).

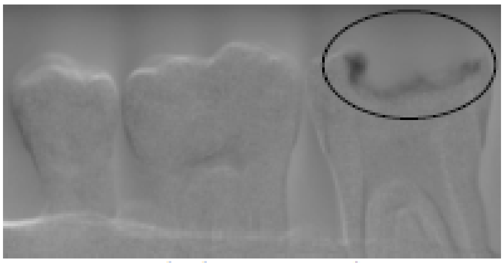

Figure 3. Subtraction image, 0° after 24 hours

The 'darker' circled area associated with the LL7 is due to the difference in grey scale values of the pixels of this area on the subtraction image compared to the rest of the tooth, following subtraction of the two digital radiographs. This 'darker' area has reduced in density and become more radiolucent compared to the rest of the tooth during the time period between taking the two digital radiographs . The 'darker' area circled therefore represents a definite area of demineralisation. In contrast there is no 'darker' area associated with the LL6. This is because there has not been any reduction in density of any area of this tooth which has occurred during the time period between taking the two digital radiographs. Therefore there is definitely no demineralisation associated with the LL6.

Each tooth will be scored using the following five-point certitude scale relating to your level of certainty as to whether or not you think there has been demineralisation of the occlusal cavity in each tooth:

1. Definitely no demineralization
2. Likely no demineralization
3. Do not know
4. Likely demineralization
5. Definite demineralization

The LL6 and LL7 in Figure 3 would score 1 and 5 respectively using the five point certitude scale above.

Variations in the angulation of x-ray projection geometry and a reduction in time of possible demineralisation will result in the production of subtraction images that are potentially more difficult to interpret. The subtraction image in Figure 4 was produced using digital radiographs at 0° after 12 hours of possible demineralisation.

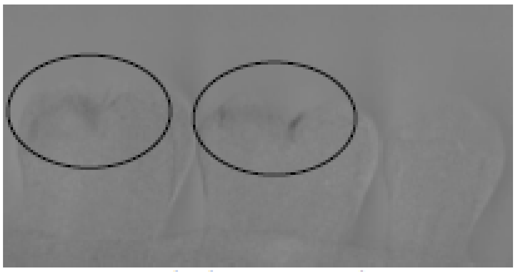

Figure 4. Subtraction Image, 0° after 12 hours

'Darker' areas associated with the LR7 and LR6 have been circled, however they are not as obvious as the 'darker' area circled on Figure 3 which was taken after 24 hours of possible demineralisation. However, the 'darker' areas circled on Figure 4 do represent a difference in grey scale values due to a reduction in density of these areas which has occurred during the time period between taking the two digital radiographs. These 'darker' areas therefore represent the presence of demineralization. The LR7 and LR6 on Figure 4 would both be scored as a 5 using the scale.

If the horizontal angulation of the x-ray beam imaging the teeth in Figure 4 is changed by 15° (Figure 5) the subtraction image becomes more difficult to interpret.

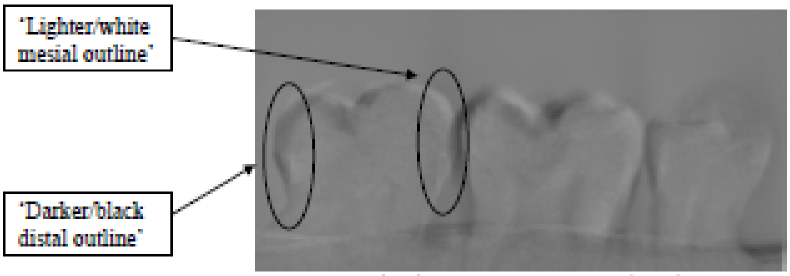

Figure 5. Subtraction Image, 15° horizontal after 12 hours

The change in horizontal angulation in Figure 5 is evident as the mesial and distal aspects of the teeth show 'darker/black' and 'lighter/white' outlines or shadows. These have been produced due to differences in the corresponding pixel grey scale values of the digital radiographs as the x-ray beam has passed through different areas of the teeth prior to hitting the phosphor digital imaging sensor. The geometrically related 'darker/black' and 'lighter/white' areas on subtraction images are useful indicators that a shift in angulation has occurred. They do not necessarily indicate the presence of demineralisation or remineralisation of a carious lesion, and in this research project it is impossible for these areas to have demineralised as the demineralising solution was only dropped into occlusal cavities.

The tooth in Figure 6 has a buccal restoration, however as the two digital radiographs used to produce the subtraction image have a reproducible x-ray projection geometry there are no differences in grey scale values of the restoration or tooth tissue immediately surrounding the restoration on the subtraction image, hence its appearance.

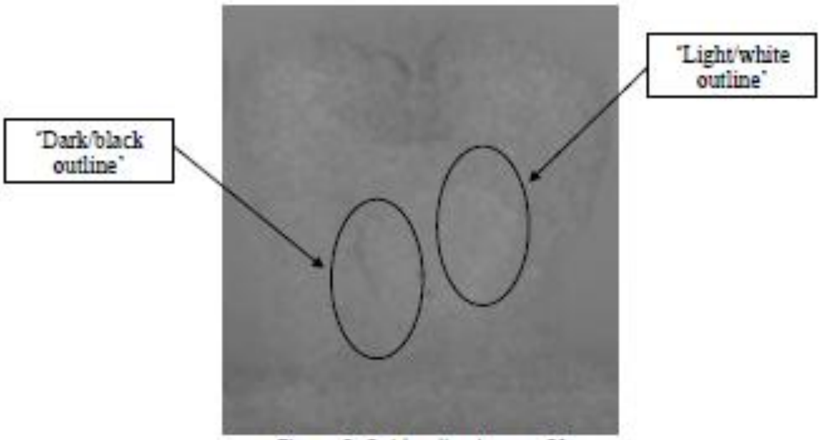

Figure 6. Subtraction image, 0°

'Dark/black' or 'light/white' outlines around restorations are however often visible even on subtraction images produced with a reproducible x-ray projection geometry due to the small distance between large variations in the grey scale values of pixels on the digital radiographs used to produce the subtraction image (Figure 6).

Changes in angulation obviously exaggerates these 'dark/black' and 'light/white' outlines creating distinct areas relative to the degree of horizontal and/or vertical difference in x-ray projection geometry. Figures 7 and 8 demonstrate the effect this has on the subtraction images produced for the same tooth as shown in Figure 6. Figure 7 has a 15° horizontal change in x-ray beam angulation and Figure 8 has a 10° vertical change in x-ray beam angulation.

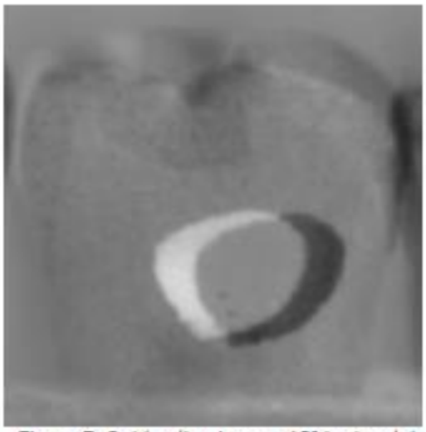
Figure 7. Subtraction image, 15° horizontal

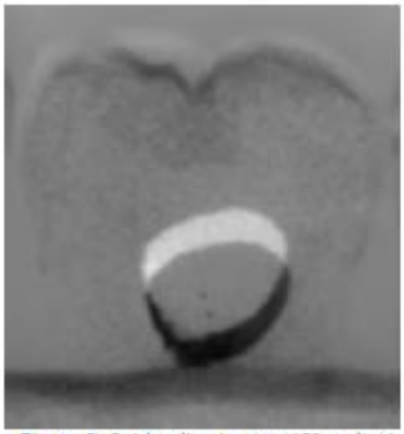
Figure 8. Subtraction image, 10° vertical

Changes in x-ray projection geometry can result in subtraction images that are difficult to interpret. Examples of this are shown in Figures 9 and 10. This reduction in level of certainty (whether or not there has been demineralisation of the occlusal cavity) would result in the images scoring 3 (Do not know) on the scale.

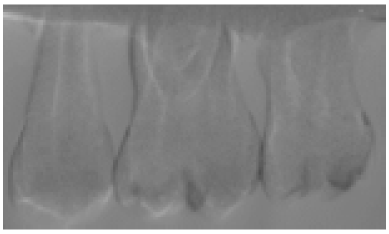

Figure 9.

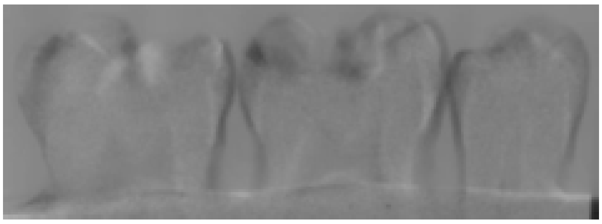

Figure 10.

When analysing the subtraction images, it is important to remember that demineralisation can only have taken place in the occlusal cavities of the molar teeth.

If you have any queries regarding analysis of subtraction images please do not hesitate to contact me, Sam Rollings via either e-mail at samrollings@nhs.net, telephone on 07877 907913 or by post sent to the Department of Restorative Dentistry, The University of Aberdeen Dental School and Hospital, Cornhill Road, Aberdeen, AB25 2ZR.

What does this CD contain?

This CD contains the following files:

'Participant Information Sheet Ver 2.doc'

- A Word document provides information regarding the research project, what would be expected of you if you agree to participate and how you should view the subtraction images.

'Subtraction Images Presentation 600.ppt'

- A PowerPoint file contains the initial 600 subtraction images to be viewed and scored by you using the five-point certitude scale.

'Data collection sheet initial 600.doc'

- Your name and the date that you finish scoring the initial 600 subtraction images should be recorded here, along with your scores for each tooth using the five-point certitude scale. Once completed, please e-mail this file to me (Sam Rollings).

'Subtraction Images Presentation Reproducibility.ppt'

- A PowerPoint file contains the 100 reproducibility subtraction images selected at random to be viewed and scored by you using the five point certitude scale one month after completion of viewing and scoring the initial 600 subtraction images.

'Data collection sheet reproducibility 100.doc'

- Your name and the date that you finish scoring the 100 reproducibility subtraction images should be recorded here, along with your scores for each tooth using the five-point certitude scale. The 100 reproducibility subtraction images should be viewed one month after finishing viewing and scoring the initial 600 subtraction images. Once completed, please e-mail this file to me (Sam Rollings).

What should I do now?

1. Please let me know if you would prefer not to be involved in the project or if you feel that you will be unable to meet the deadlines of Sunday 29th April 2012 for the scoring of the initial 600 subtraction images and Sunday 27th May 2012 for the scoring of the 100 reproducibility subtraction images.

2. Please view and score the initial 600 subtraction images and e-mail me your completed Word document ensuring that your name, the date you finished scoring the images and your scores are included.

Remember, when you look at each tooth you are being asked to record your level of certainty using the five point certitude scale as to 'whether or not there has been demineralisation of the occlusal cavity in that tooth'. No other areas of the teeth will have demineralised as demineralising solution was only applied to the occlusal cavities prepared in 19 of the 40 molar teeth which were selected at random.

3. I will e-mail you one month after the date you finished scoring the initial 600 images to prompt you to view and score the 100 reproducibility images and e-mail me your completed Word document ensuring that your name, the date you finished scoring the images and your scores are included.

Your scores will then be entered into a database for statistical analysis and you will be kept informed of the results. You will also be kept informed of potential submission of an article/articles for publication using this data.

Many thanks again for agreeing to participate with this research project. If you have any questions regarding any aspect of it please do not hesitate to contact me.

Kind regards

Sam Rollings

REFERENCES:

RICKETTS, D. N. J., EKSTRAND, K. R., MARTIGNON, S., ELLWOOD, R., ALATSARIS, M. & NUGENT, Z. 2007. Accuracy and reproducibility of conventional radiographic assessment and subtraction radiography in detecting demineralization in occlusal surfaces. *Caries Research*, 41, 121-128.

9.4 Appendix IV

Data collection sheet – 600 Images

Examiner's name	
Date	

Evidence of Lesion Progression	
Level of certainty	**Score**
Definitely no progression	1
Likely no progression	2
Do not know	3
Likely progression	4
Definite progression	5

PowerPoint Slide Number	Tooth	Evidence of Lesion Progression (enter score of 1-5)
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		

9.5 Appendix V

Data collection sheet – Reproducibility

Examiner's name	
Date	

Evidence of Lesion Progression	
Level of certainty	Score
Definitely no progression	1
Likely no progression	2
Do not know	3
Likely progression	4
Definite progression	5

PowerPoint Slide Number	Tooth Number	Evidence of Lesion Progression (enter score of 1-5)
1		
2		
3		
4		
5		
6		
7		
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9		
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11		
12		
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14		
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19		

9.6 Appendix VI

ACCURACY AND REPRODUCIBILITY OF ASSESSING DIFFERENCES BETWEEN DIGITAL BITEWING RADIOGRAPHS GENERATED BY CHANGES IN THE HORIZONTAL ANGULATION OF THE X-RAY PROJECTION GEOMETRY

PARTICIPANT INFORMATION SHEET

The changing philosophy in caries management has seen a move away from complete surgical excision to more conservative approaches involving incomplete caries removal and sealing the carious biomass within the tooth. These approaches are based upon the microbiology and histopathology of the lesions and pulp-dentine complex reactions. The aim of this approach is to arrest lesion progression and hence preserve pulpal health. However, once sealed, it can be difficult to assess whether lesion progression has been arrested or not and hence the success of the management technique.

Early research by Handelman *et al.* [1] suggested that fissure sealants could be used to manage carious lesions that were radiographically visible on the occlusal surface using bitewing radiographs at recall to monitor lesion arrest/progression. However, more recent in vitro research by Ricketts *et al.* [2] has demonstrated that digital subtraction radiography is more reproducible and accurate in detecting occlusal demineralization than viewing paired digital radiographic images side by side. In this study, a reproducible X-Ray projection geometry was used which produces digital subtraction images of high diagnostic quality.

Digital subtraction radiography software can however overcome differences in digital radiographs that have been generated due to changes in angulation of the X-Ray projection geometry. My recent research project revealed that accurate and reproducible assessment of occlusal demineralization using digital subtraction images was possible using digital radiographs that had been generated with up to a 7 degree horizontal difference in X-Ray projection geometry. Any difference in vertical angulation or difference in horizontal angulation of 15 degrees or more statistically significantly reduced both the accuracy and reproducibility of the ability to assess occlusal demineralization.

In clinical practice it is difficult, if not impossible to accurately calculate what angulation difference exists in X-Ray projection geometry between two digital radiographs. The use of intra-oral film holders with beam aiming devices and bite blocks that can be customized for individual patient use can help standardize X-Ray projection geometry. However, angulation differences in X-Ray projection geometry may still occur and if you have not taken the radiographs you may not know if attempts have been made to standardize the X-Ray projection geometry.

I would like to invite you to take part in this addition element of my *in-vitro* research project which aims to assess the accuracy and reproducibility of a scoring system for assessing differences in horizontal angulation of X-Ray projection geometry used to produce digital bitewing radiographs.

What will I have to do if I take part?

Participation in this additional element of my research project will involve looking at digital radiographs and grading the inter-proximal spaces or contact points between teeth using a scoring system.

How long will it take?

Sixty digital radiographs have been produced and it would take approximately 30 minutes to look at all the images. To enable assessment of intra-examiner variability you will also be required to look at the same 60 digital radiographs in a different order generated at random two weeks later.

What are the possible risks of taking part?

The digital radiographs will be provided in a Microsoft PowerPoint file which can be viewed on any PC. The digital radiographs should be viewed while you are sitting in a comfortable position with the monitor the correct height and distance from your eyes to prevent strain.

Are there any possible benefits?

Participation in this research project will improve your familiarity viewing digital radiographs. It is envisaged that the results from this additional element of my research project will be submitted for publication in an appropriate peer reviewed journal. As you will have been involved with the acquisition of data your name will be included as an author if you provide final approval of the article (following any necessary drafting and revision) for publication.

Do I have to take part?

No, taking part is voluntary. If you would prefer not to take part you do not have to give a reason.

What happens to the information that you provide?

The University of Dundee complies with the Data Protection Act and all information will be treated with the strictest confidence.

Funding

This additional element of my research project has not received any funding.

Ethics approval

The East of Scotland Research Ethics Service has determined that this research project does not require ethical approval.

Can I get any more information?

If you have any other queries please feel free to contact the research team at the address, or via the telephone number or e-mail address given below:

Research Team

Sam Rollings (David Ricketts, Nicola Innes)

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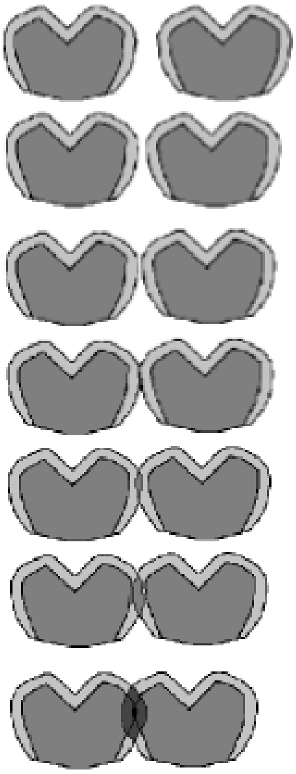
References:

1. Handelman, S.L., F. Washburn, and P. Wopperer, *Two-year report of sealant effect on bacteria in dental caries.* J Am Dent Assoc, 1976. **93**(5): p. 967-70.
2. Ricketts, D.N., et al., *Accuracy and reproducibility of conventional radiographic assessment and subtraction radiography in detecting demineralization in occlusal surfaces.* Caries Res, 2007. **41**(2): p. 121-8.

9.7 Appendix VII

Overlap Assessment – Initial 60

Examiner Name:		Date:	



Score = -3
Inter-proximal space greater than the width of enamel of one tooth

Score = -2
Inter-proximal space less than the width of enamel of one tooth but greater than half the width of enamel of one tooth

Score = -1
Inter-proximal space less than half the width of enamel of one tooth

Score = 0
No space between or superimposition of contact points

Score = +1
Superimposition of contact points into the outer half of enamel

Score = +2
Superimposition of contact points into the inner half of enamel but not into dentine

Score = +3
Superimposition of contact points into dentine

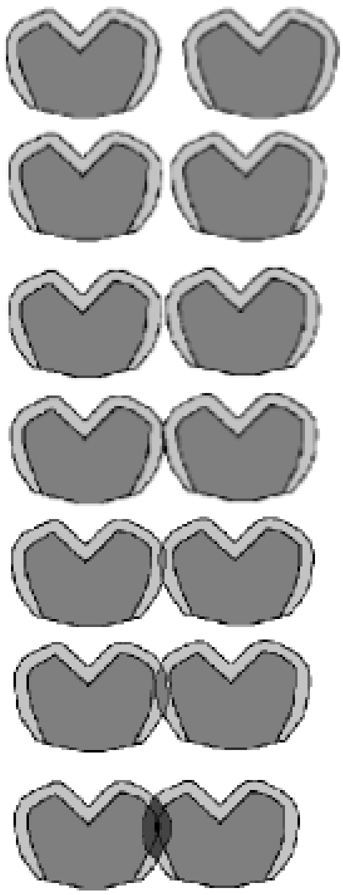
Powerpoint Slide Number	I-P Contact	Score
12	5/6	
	6/7	
9	5/6	
	6/7	
22	5/6	
	6/7	
15	5/6	
	6/7	
48	5/6	
	6/7	
7	5/6	
	6/7	
45	5/6	
	6/7	
29	5/6	
	6/7	
11	5/6	
	6/7	
54	5/6	
	6/7	
24	5/6	
	6/7	
47	5/6	
	6/7	
19	5/6	
	6/7	
51	5/6	
	6/7	
25	5/6	
	6/7	
60	5/6	
	6/7	
17	5/6	
	6/7	
44	5/6	
	6/7	
49	5/6	
	6/7	
42	5/6	
	6/7	
52	5/6	
	6/7	

9.8 Appendix VIII

Overlap Assessment – Reproducibility

-To be completed 2 weeks after initial scoring-

Examiner Name:		Date:	



Score = -3
Inter-proximal space greater than the width of enamel of one tooth

Score = -2
Inter-proximal space less than the width of enamel of one tooth but greater than half the width of enamel of one tooth

Score = -1
Inter-proximal space less than half the width of enamel of one tooth

Score = 0
No space between or superimposition of contact points

Score = +1
Superimposition of contact points into the outer half of enamel

Score = +2
Superimposition of contact points into the inner half of enamel but not into dentine

Score = +3
Superimposition of contact points into dentine

Powerpoint Slide Number	I-P Contact	Score
48	5/6	
	6/7	
38	5/6	
	6/7	
58	5/6	
	6/7	
60	5/6	
	6/7	
26	5/6	
	6/7	
30	5/6	
	6/7	
27	5/6	
	6/7	
4	5/6	
	6/7	
10	5/6	
	6/7	
14	5/6	
	6/7	
55	5/6	
	6/7	
11	5/6	
	6/7	
8	5/6	
	6/7	
56	5/6	
	6/7	
20	5/6	
	6/7	
50	5/6	
	6/7	
5	5/6	
	6/7	
9	5/6	
	6/7	
22	5/6	
	6/7	
19	5/6	
	6/7	
53	5/6	
	6/7	